University of Alberta

Glutamate and Dopamine in the Rat Nucleus Accumbens: Effects on Locomotor Activity and Reward

by

Kwang-Ho Choi ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Division of Neuroscience

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ABSTRACT

Interactions between dopamine and glutamate in the nucleus accumbens (NAS) of the mesolimbic system may play a significant role in the pathophysiology of schizophrenia and drug addiction. The purpose of this study was to investigate the effects of dopamine- and glutamate-related compounds on behavioural and neurochemical responses in rats. Glutamate receptor agonists or antagonists in combination with dopamine receptor agonists were administered directly into the rat NAS core and shell; spontaneous locomotor activity, brain stimulation reward and extracellular dopamine and glutamate levels were measured. Major findings from these experiments were: (1) Intra-NAS administration of a glutamate uptake inhibitor PDC elevated extracellular glutamate levels but did not alter locomotor activity; (2) Chronic oral administration of the NMDA receptor co-agonist glycine increased brain glycine levels, but did not alter brain stimulation reward thresholds; (3) The AMPA/kainate receptor antagonists CNQX and NBQX potentiated the locomotor suppressant effect of the dopamine D2/D3 receptor agonist 7-OH-DPAT when co-administered into the NAS core or shell; (4) Co-administration of CNQX and 7-OH-DPAT into the NAS core or shell increased brain stimulation reward thresholds; and (5) Co-administration of the dopamine D1 receptor agonist SKF 38393 and the D2/D3 receptor agonist quinpirole into the NAS shell, but not into the core, induced a synergistic locomotor stimulation. The present results suggest that simultaneous stimulation of dopamine D2/D3 receptors and blockade of AMPA/kainate receptors in the NAS core and shell may synergistically reduce locomotion and brain stimulation reward. In addition, stimulation of both D1 and D2/D3

dopamine receptors in the NAS shell may be necessary to induce synergistic locomotor stimulation. These results contribute to our understanding of the role of dopamine-glutamate interactions in the mesolimbic system in regulating movement and reward-related behaviour.

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ABBREVIATIONS

5-HIAA	5-hydoxyindole-3-acetic acid
5-HT	5-hydroxytryptamine
7-OH-DPAT	(±)-2-dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene
AMPA	2-amino-3-(3-hydroxy-5-methyl-4-isoxazolo)-propionic acid
AMPH	(+)-amphetamine
ANOVA	analysis of variance
AP	anterior-posterior
AP-5	amino-5-phosphonopentanoic acid
CNQX	6-cyano-7-nitro-1,2,3,4-tetrahydro-quinoxaline-2,3-dione
CPP	(±)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid
CSF	cerebrospinal fluid
DNQX	6,7-dinitroquinoxaline-2,3-dione
DOPAC	3,4-dihydroxyphenylacetic acid
DV	dorsal-ventral
GABA	γ-aminobutyric acid
GYKI 52466	1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5H-2,3-benzodiazepine
HBC	2-hydroxypropyl-β-cyclodextrin
HPLC	high pressure liquid chromatography
HVA	homovanillic acid
i.p.	intraperitoneal
IC_{50}	concentration producing 50% inhibition of binding

ICSS	intracranial self-stimulation
M50	a frequency that maintains half-maximal response rates
MK-801	(±)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine
ML	medial-lateral
NAS	nucleus accumbens
NBQX	2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide
NMDA	N-methyl-D-aspartic acid
p.o.	per os, orally
PCP	phencyclidine
PDC	L-*trans*-pyrrolidine-2,4-dicarboxylate
RMAX	maximal number of responses at a single frequency
s.c.	subcutaneous
SKF 38393	1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol
TRES	total number of responses per session
TTX	tetrodotoxin
VTA	ventral tegmental area
ZFRE	a frequency at which response rates dropped to zero

1. GENERAL INTRODUCTION

The monoamine neurotransmitter dopamine and the excitatory amino acid glutamate in the brain may play a significant role in the control of movement and goal-directed behaviour. Within the ventral part of the forebrain, the nucleus accumbens (NAS) receives dopaminergic and glutamatergic inputs from the ventral tegmental area and the prefrontal cortex, respectively. Abnormal interactions between dopamine and glutamate in the NAS have been implicated in the pathophysiology of schizophrenia and the mechanism of drug addiction.

1.1. Dopamine and Glutamate Hypothesis of Schizophrenia

Schizophrenia is characterized by positive symptoms such as delusions, hallucinations, disorganized speech and catatonic behaviours as well as negative symptoms such as flat affect, cognitive problems and poor social interactions (Diagnostic and Statistical Manual of Mental Disorders IV). The onset of schizophrenia usually occurs during late adolescence or early adulthood, and there is a prevalence rate of 1% in the general population worldwide. Approximately 50% of schizophrenic patients attempt suicide and 15% of them succeed. Despite the notable studies that have been made in the past 30 years, the neuropathology of schizophrenia is still not well understood. Structural abnormalities of the brain, including ventricular enlargement, decreased cortical and hippocampal volumes and neurochemical dysfunction involving dopamine or 5-hydroxytryptamine or glutamate have been proposed in the neuropathology of

schizophrenia (Knable et al. 1995; Harrison 1999). The neurochemical abnormalities involving dopamine and glutamate are summarized in this section.

1.1.1. Dopamine Hypothesis of Schizophrenia

The proposal that excessive dopamine activity or unusually high responses to dopamine in the mesolimbic dopamine system of the brain remains the dominant neurochemical hypothesis for schizophrenia (Joyce 1993). There are several lines of evidence supporting this hypothesis. Chronic use of the psychostimulant amphetamine, which is an indirect dopamine receptor agonist, can induce psychotic symptoms in humans (Connell 1958; Randrup and Munkvad 1965). In addition, many antipsychotic drugs block D2 dopamine receptors in the brain (Creese et al. 1976; Seeman et al. 1976). Post-mortem studies also indicate that D2 dopamine receptors in the mesolimbic system are increased in schizophrenic patients (Owen et al. 1978; Hyde et al. 1991). However, this latter effect may have been attributed to chronic medication with antipsychotics which have marked effects on D2-like (D2, D3 and D4) dopamine receptors (see Reynolds and Czudek 1995). Partly on the basis of these observations, it has been suggested that dopamine hyperactivity in the mesolimbic system may cause positive symptoms such as hallucinations, paranoia and catatonia. However, the dopamine hypothesis alone may not be sufficient to explain either the lack of effect of most antipsychotics on negative symptoms including flat affect, poor social interaction and cognitive dysfunction (Carpenter 1996; Kinon and Lieberman 1996) or the differential temporal responses between drug action at the dopamine receptors and clinical improvement in schizophrenic patients (Marder and Van Putten 1995).

1.1.2. Glutamate Hypothesis of Schizophrenia

In addition to dopaminergic hyperactivity, abnormal glutamate function in the forebrain has also been suggested in schizophrenia. It has been reported that glutamate levels in cerebrospinal fluid are 50% lower in schizophrenics relative to normal subjects (Kim et al. 1980), although other studies (Perry 1982; Korpi et al. 1987) are not consistent with this finding. Abnormal function of glutamate receptors in the brain may be involved in the pathophysiology of schizophrenia. In radioligand binding studies (Toru et al. 1988; Deakin et al. 1989; Simpson et al. 1992), N-methyl-D-aspartic acid (NMDA) receptors were increased in the frontal cortex of schizophrenic patients, although antipsychotic-medicated or non-medicated patients were not well distinguished in these studies. NMDA receptor antagonists, such as PCP, MK-801 and ketamine, may induce psychotic symptoms in normal humans and schizophrenic patients (Javitt and Zukin 1991; Coyle 1996). Based on these findings, decreased NMDA receptor function has been suggested in the pathophysiology of schizophrenia (Olney and Farber 1995).

Glycine therapy may be beneficial in improving schizophrenic symptoms based on the NMDA receptor hypofunction hypothesis. The amino acid glycine is a co-agonist at a strychnine-insensitive site on the NMDA receptor in the brain (Johnson and Ascher 1987; Kleckner and Dingledine 1988). Some clinical studies have reported improvement of schizophrenic symptoms using agonists at the glycine site such as glycine, D-serine and D-cycloserine (Goff et al. 1995; Heresco-Levy et al. 1996; Tsai et al. 1998). Chronic oral administration of glycine may improve the negative symptoms of schizophrenia (Heresco-Levy et al. 1996) and this effect may be mediated by mildly enhancing NMDA

receptor function in the brain (Farber et al. 1999). In contrast, other studies reported a lack of clinical efficacy of glycine and D-cycloserine (D'souza et al. 2000) and furthermore, possible neurodegenerative effects of glycine in the brain (Waziri and Baruah 1999).

Non-NMDA glutamate receptors, such as 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolo)-propionic acid (AMPA) receptor, may also play a significant role in schizophrenia. Expression of the AMPA receptor subtype GluR1 is decreased in the medial temporal lobe of schizophrenic patients (Harrison et al. 1991). Eastwood et al. (1997) reported that GluR1 and GluR2/3 are decreased in the hippocampal regions of schizophrenic patients. These findings are not likely attributed to drug effects because antipsychotic medication either did not change or increased the AMPA receptor expression in these regions (Oretti et al. 1994; Fitzgerald et al. 1995). In animal studies, AMPA receptors have been reported to have differential influences on mesolimbic dopamine function relative to NMDA receptors (Karler et al. 1991; Hauber and Andersen 1993; Bubser et al. 1995). Hauber and Andersen (1993) reported that the AMPA/kainate receptor antagonist GYKI 52466 attenuated locomotor stimulation induced by the NMDA receptor antagonist MK-801.

In relation to the dopamine hypothesis, abnormal interactions between dopamine and glutamate have been suggested in the pathophysiology of schizophrenia (Carlsson and Carlsson 1990; Grace 1992; Toru et al. 1994; Csernansky and Bardgett 1998). Carlsson and Carlsson (1990) suggested that decreased glutamatergic inputs from the hippocampus and other limbic structures to the NAS may result in psychotic symptoms. Grace (1992) suggested that a decrease in glutamate input to the NAS may increase

phasic dopamine release *via* reduction of tonic dopamine release, resulting in a reduction of the activation of dopamine autoreceptors that regulate the phasic dopamine release. Toru et al. (1994) suggested that increased mesolimbic dopamine activity leads to reduced glutamate neurotransmission in the frontal cortex, which may result in negative symptoms of schizophrenia. Furthermore, Csernansky and Bardgett (1998) proposed that schizophrenia may be due to the limbic-cortical neuronal damage caused by abnormal mesolimbic dopamine and corticolimbic glutamate states. Therefore, the dopamine and glutamate hypotheses of schizophrenia have provided the basis for studying dopamine- and glutamate-related compounds in animals. Several animal models of schizophrenia have been developed to study the effects of potential antipsychotic drugs.

1.1.3. Animal Models of Schizophrenia

A goal of using appropriate animal models in psychiatric research is to predict the clinical efficacy of potential drugs. This represents predictive validity, which is defined as "the ability of a test to predict a criterion that is of interest to the investigator" (Cronbach and Meehl 1955). Due to the presence of cognitive and language dysfunction in schizophrenic patients, it is difficult to construct animal models of schizophrenia. However, several animal models may be useful to predict the clinical efficacy of potential antipsychotics.

Locomotor hyperactivity induced by the psychostimulant amphetamine can be used as an animal model of schizophrenia (Iversen 1986; Josselyn and Vaccarino 1998). Locomotor activation mediated by direct injection of amphetamine into the NAS may be a measure of mesolimbic dopamine function. Iversen (1986) suggested that antipsychotic

drug effects at the NAS may be considered as reflecting antipsychotic efficacy while effects at the striatum are considered to reflect extrapyramidal side effects. It has been reported that many antipsychotic drugs reduce the locomotor stimulant effect of amphetamine (Costall et al. 1990). However, this animal model is mainly based on the dopamine hypothesis of schizophrenia and hyperactivity is not usually observed in schizophrenic patients (see Reynolds and Czudek 1995).

Another animal model is electrical brain self-stimulation in rats. Direct electrical stimulation of specific brain regions can establish habit-forming behaviour in animals similar to those of natural rewards such as food or sexual contact (Wise 1996a). Administration of antipsychotic drugs may induce an inhibition of electrical self-stimulation behaviour (Josselyn et al. 1997). This animal model can be used to distinguish effects of drug on motor performance and reward, which are relevant for predicting extrapyramidal side effects and antipsychotic efficacy, respectively. Using threshold analysis with operant behaviour (lever pressing), rate-free reward threshold can be dissociated from response rate in rats (see Liebman 1989).

Prepulse inhibition of startle reflexes is another animal model of schizophrenia based on an hypothesized "sensorimotor gating" mechanism. A startle reflex is a skeletomuscular response to sudden and intensive stimuli and is usually classified as a defensive reaction (Davis 1984). If a weak prestimulus such as a low-intensity tone is presented shortly before the startle stimulus, the magnitude of the startle reflex decreases. This prepulse inhibition is diminished in schizophrenic patients (Braff et al. 1992) and antipsychotic drugs reverse this reduced prepulse inhibition (Swerdlow et al. 1994). Prepulse inhibition may also be disrupted by administration of dopamine receptor

agonists, glutamate receptor antagonists and by surgical manipulation of areas of the limbic system, including the limbic cortex, striatum, ventral pallidum and pontine reticular formation in rats (Swerdlow and Geyer 1998).

1.2. The Nucleus Accumbens (NAS)

1.2.1. Neuronal Pathways in the NAS

The NAS, located in the ventral striatum of the mammalian forebrain, has a unique set of inputs from most of brain regions that have been implicated in schizophrenia. The NAS receives ascending "mesolimbic" dopamine input from the ventral tegmental area (VTA) and descending "corticolimbic" glutamate input from the prefrontal cortex. It has been proposed that the NAS may play a significant role in the integration and further processing of information related to motivation and motor output (Mogenson et al. 1980; Mogenson and Yang 1991). The NAS receives major afferents from the VTA, the prefrontal cortex, basolateral amygdala, hippocampus, paraventricular nucleus and parvoventricular nucleus of the thalamus (Figure 1.1). The NAS sends major efferents to several regions such as the VTA, the globus pallidus, the preoptic area, the lateral hypothalamus, the dorsomedial thalamus and the paraventricular nucleus (Figure 1.2).

1.2.2. Dopamine Receptors in the NAS

Dopamine receptors are classified into two major subfamilies: D1-like and D2-like dopamine receptors (see Seeman 1995; Neve and Neve 1997). D1-like receptors are

comprised of D1 and D5 receptor subtypes and D2-like receptors include D2, D3 and D4 receptor subtypes. The D1-like receptors and D2-like receptors stimulate and inhibit adenylyl cyclase respectively (Civelli et al. 1993). Within the central nervous system, dopamine neurons have three main projection pathways, namely the mesocortico/mesolimbic, the nigrostriatal and the tuberoinfundibular pathways. Dopamine receptors, especially D1 and D2 subtypes, are found in both presynaptic and postsynaptic locations in the NAS. Within the NAS, D2 receptors are more abundant in the NAS core whereas the concentration of D1 and D3 receptors is relatively higher in the NAS shell than the core (Bardo and Mammer 1991; Jongen-Relo et al. 1995; Le Moine and Bloch 1996). In terms of dopamine receptor pharmacology, there are highly selective agonists and antagonists that can distinguish between D1-like and D2-like receptors (see Sibley 1999). However, few agents are selective for individual subtypes within the D1-like and D2-like receptor families. Therefore, given the limited selectivity of these compounds, it is difficult to reliably stimulate or inhibit each subtype within the two dopamine receptor subfamilies (D1 vs. D5 or D2 vs. D3) in intact animals. The dopamine receptors are summarized in the Table 1.1.

1.2.3. Glutamate Receptors in the NAS

Glutamate receptors are classified into ionotropic and metabotropic receptor families. Both ionotropic and metabotropic glutamate receptors have been reported to be present in the NAS. Ionotropic glutamate receptors are distinguished by specific binding of the agonists NMDA, AMPA and kainic acid (Dingledine et al. 1999). NMDA receptors usually co-exist with AMPA receptors in a single synapse and these receptors

are involved in amplification of glutamate signals. Because NMDA receptors are normally blocked by Mg^{2+} at resting potential, depolarization of the postsynaptic membrane by AMPA or kainate receptor activation is necessary to enable direct ligand activation of NMDA receptors. AMPA receptors are classified into GluR1-GluR4 subtypes (Dingledine et al. 1999). Metabotropic glutamate receptors, in contrast to the ionotropic receptors, are linked with second messenger systems such as phosphoinositide and cyclic nucleotides (e.g. cAMP) (Pin and Duvoisin 1995). The metabotropic glutamate receptor family comprises eight subtypes (mGlu1-8), subdivided into three groups (group I-III) based on sequence similarity and transduction pathways. Group I (mGlu1 and mGlu5) receptors activate phospholipase C, whereas group II (mGlu2 and mGlu3) and group III (mGlu4, mGlu6, mGlu7 and mGlu8) receptors inhibit adenylyl cyclase activity (Conn and Pin 1997). These authors suggested that group I receptors are mostly located in postsynaptic membranes which increase cell excitability while group II and group III receptors are mainly located on glutamate terminals and inhibit glutamate release. Ionotropic glutamate receptors are summarized in Table 1.2.

1.2.4. Heterogeneity of the NAS

Previous studies have suggested that the NAS has two major functional sub-regions, the core and shell, based on immunoreactivity for calcium-binding protein (Heimer et al. 1991; Zahm and Brog 1992). It has been suggested that the NAS shell may play a primary role in limbic function, whereas the core may be more involved in motor system function (Deutch et al. 1993). The input and output connections are different between the NAS core and shell sub-regions. For example, the NAS core and shell

regions project to the pallidal areas with a distinct topography. The NAS core projections are located in the dorsolateral part of ventral pallidum, whereas the shell projects to the medial part of the ventral pallidum (Groenewegen et al. 1999). The core and shell also receive different combinations of inputs from the prefrontal cortex, amygdala, thalamus, hippocampus and the VTA/substantia nigra (Figure 1.3). This distinction within the NAS is of interest in view of contrasting reports of dopamine-dependent (Svensson et al. 1994a; Moghaddam and Bolinao 1994) and dopamine-independent (Druhan et al. 1996; Balfour et al. 1996) effects of glutamate receptor-related compounds on neurochemistry and behaviour.

1.3. Dopamine and Glutamate Interactions in the NAS

It is well established that the NAS receives both mesolimbic dopaminergic and corticolimbic glutamatergic inputs. In the NAS, dopamine and glutamate terminals are found to exist on a postsynaptic medium spiny neuron in what has been described for the cortex as a "synaptic triad" arrangement (Goldman-Rakic et al. 1992). This model suggests that the dendritic spine of the postsynaptic neuron is closely juxtaposed to two neuronal terminals, containing dopamine and exhibiting the asymmetric structure typical of glutamate synapses, respectively. A number of studies suggest a reciprocal interaction between dopamine and glutamate in the NAS (see Morari et al. 1998). For example, respective modulation of dopamine release by glutamate (Imperato et al. 1990) and of glutamate transmission by dopamine (Yang and Mogenson 1987) have been reported.

1.3.1. Locomotor Activity

Both dopamine and glutamate receptors in the NAS may play a role in the control of locomotor activity. Administration of the NMDA receptor antagonist AP-5 into the NAS increased locomotor activity in "monoamine-depleted" mice (Svensson and Carlsson 1992). This study suggests that NMDA receptor antagonist may induce behavioural stimulation independent of dopamine in the NAS. However, Kelley and Throne (1992) reported that intra-NAS administration of AP-5 decreased locomotor activity in amphetamine-stimulated rats. Others reported the effects of AMPA/kainate receptor antagonists on locomotor activity. Administration of DNQX or CNQX into the NAS attenuated amphetamine-induced hyperactivity (Willins et al. 1992; Burns et al. 1994). Kaddis et al. (1993) demonstrated that intra-NAS administration of DNQX inhibited hyperactivity induced by cocaine or a mixed D1 and D2 dopamine agonist (SKF 38393 and quinpirole). Boldry et al. (1991) reported that administration of AMPA into the NAS increased locomotor activity and either D1 antagonist SCH 23390 or D2 antagonist sulpiride inhibited this hyperactivity.

1.3.2. Reward

Interactions of dopamine and glutamate in the mesolimbic system may play a role in motivation and reward-related behaviour. Systemic administration of the NMDA receptor antagonist MK-801 facilitated the lateral hypothalamic self-stimulation in rats (Olds 1996). Although this study used simple response rate measures which make it difficult to dissociate the reward and motor performance, it is interesting that the dopamine antagonists haloperidol and SCH 23390 attenuated the effects of MK-801 in

that study. This observation may provide evidence for a dopaminergic basis of the facilitatory effect of MK-801 on electrical self-stimulation.

Carlezon and Wise (1996a) reported that the NMDA receptor antagonists PCP and MK-801 and the dopamine uptake blocker nomifensine increased VTA self-stimulation reward when these drugs were administered directly into the NAS shell. In a drug self-administration study in which animals' operant responses are reinforced by drug-infusions into the NAS, the NMDA receptor antagonists PCP, MK-801 and CPP, as well as the dopamine uptake inhibitors cocaine and nomifensine were self-administered into the NAS shell (Carlezon and Wise 1996b). These results indicate that either NMDA receptor antagonism or dopamine uptake blockade in the NAS may have reward enhancing effects.

Pairing one distinct but neutral environment with a rewarding stimulus and another neutral environment with a non-rewarding stimulus can induce a conditioned place preference in animals. Animals subsequently given a chance to spend time in both environments exhibit a conditioned place preference for an environment previously associated with a rewarding stimulus (Spyraki et al. 1982). Tzschentke and Schmidt (1995) found that systemic administration of MK-801 blocked morphine-induced conditioned place preference, while this compound had no rewarding effect when given alone. Administration of AMPA/kainate receptor antagonist DNQX into the NAS inhibited acquisition of conditioned place preference induced by amphetamine (Layer et al. 1993). In a similar study, Kaddis et al. (1995) reported that intra-NAS administration of DNQX inhibited acquisition of cocaine-induced conditioned place preference. Taken together, these studies suggest that glutamate receptor antagonists, especially

AMPA/kainate receptor antagonists, may reduce conditioned reward induced by psychostimulant drugs such as cocaine and amphetamine.

1.3.3. Differential Effects in the NAS Core and Shell

The NAS core and shell may have functional differences in response to dopamine receptor- and glutamate receptor-mediated behaviour (Zahm 1999). For example, administration of the NMDA receptor antagonist AP-5 into the NAS shell increased spontaneous locomotor activity, while administration into the core decreased locomotion (Maldonado-Irizarry and Kelley 1994). This compound also reduced cocaine-induced hyperactivity when microinjected into the NAS core (Pulvirenti et al. 1994). Administration of AMPA into the NAS core induced a greater increase in locomotor activity than that observed following administration of AMPA into the shell (Johnson et al. 1996). In addition, administration of CNQX into the NAS core reduced locomotor stimulation induced by cocaine sensitization (Pierce et al. 1996). In another study related to the area of motivation and reward, Kelley (1999) reported that administration of CNQX or NBQX into the shell, but not into the core, induced feeding behaviour in satiated rats. Carlezon and Wise (1996a) reported that PCP and MK-801 increased brain stimulation reward when these compounds were microinjected into the NAS shell. Based on these studies, it is likely that the NAS shell is preferentially involved in the role of reward-related behaviour whereas the core is related to motor system function.

1.4. Methodology

1.4.1. Microinjection and Locomotor Activity

Intracranial microinjection refers to the application of a drug directly into brain tissue. A common experimental procedure is the permanent placement of a cannula into a specific region of the brain. Less than a microliter volume of drug solution can be injected into the brain to determine behavioural responses (see Greenshaw 1985). There are several advantages to using the intracranial microinjection technique over systemic drug injection. It is possible to test the involvement of specific neurotransmitter receptors in the specific brain region by injecting drug into the target area. In the case of systemic administration, the net effect of a drug on behaviour may reflect multiple actions in different brain areas that may oppose each other. Intracranial microinjection can also prevent the peripheral effects of the parent drug and its metabolites. Moreover, high local concentrations of drug in the brain following microinjection can affect different sub-populations of receptors that are not affected by systemic injection of drugs due to poor permeability of the blood-brain barrier system (Wolf 1998).

Spontaneous locomotor activity may be used for global assessment of stimulant or depressant effects of drugs. Locomotor activity can be monitored by automatic measures using the interruptions of infrared photobeams between a light source and a light-activated switch on the opposite side of an activity box (see Dourish 1987). Interruption of the beam operates a pulse-forming circuit that activates a digital counter. Activity scores from a series of counters can be monitored automatically by a microcomputer system for temporal analysis. The photobeam activity boxes used in the present study

have 12 × 12 horizontal photobeams with another 12 photobeams attached to the cage walls to measure vertical activity. Total horizontal activity, including locomotion, grooming, scratching, head swaying and tail movements, is determined by the interruption of any one of the 12 × 12 photobeams. In addition, stereotypic behaviour was determined by the consecutive interruption of the same photobeams.

When monitoring effects of drugs on locomotor activity, determination of baseline activity may be important because of possible floor effects (sedative effect of drug on low baseline activity) or ceiling effects (stimulant effect of drug on high baseline activity). These baselines can be adjusted using non-habituation or different habituation protocols such as daily exposure to the test environment or exposure of the animal to the environment for a short period prior to behavioural testing. However, one of the major limitations of measuring spontaneous locomotor activity is that locomotor activity is not a single homogenous behavior (Kelley et al. 1989). It is a complex set of behaviors consisting of a variety of components, including walking, rearing, turning, sniffing and grooming.

1.4.2. Electrical Brain Self-stimulation

Electrical brain self-stimulation is an artificial form of reward. Animals readily engage in lever-pressing behaviour that is reinforced by electrical stimulation to certain brain areas (Olds and Milner, 1954). Electrical brain self-stimulation has been widely used to study drug effects on reward-related behaviour and to screen potential antipsychotic drugs. Several brain areas are identified as targets for electrical self-

stimulation, including the orbitofrontal cortex, the NAS, the septum, lateral hypothalamus, medial forebrain bundle and the VTA (see Rolls 1999).

Edmonds and Gallistel (1974) introduced a parametric study of electrical self-stimulation which manipulated one of the stimulus parameters, enabling more precise control of the experiment than when natural rewards were used. Under this procedure, an animal is run between a start box in which it receives "priming" stimulation, and a goal box at the other end of an alley, where it has access to rewarding lever-pressing. By independently varying the stimulation parameters, the changes in running speed can be measured. The running speed is low at short pulse trains, but this speed suddenly increases to an asymptote as train length is increased. The location of sharp rise in running speed can be determined as a reward value.

Two types of responses can be measured with operant behaviour: rate and threshold measures (see Liebman 1989; Greenshaw and Wishart 1987). A typical rate measure is simply the number of lever-press responses which has often been used due to high numbers of responses generated in animals. However, response rate is not necessarily related to reward. Hodos and Valenstein (1962) reported that response rate may not be correlated with the site of preference when different stimulation sites were compared. In the same rats, stimulation of the septum generally maintained lower response rates than hypothalamic stimulation over a range of current intensities. However, when the animals were allowed to self-stimulate at either site, septal stimulation was preferred to hypothalamic stimulation. In addition, the influences of drugs on response rate may be attributed to a wide range of effects: stimulant or sedative effects, or changes in attentional or memory process (Iversen 1977). In contrast to rate

measures, threshold measures may be more sensitive to reward value and are considered to be relatively independent of rate of responding. The most widely accepted "rate-free" threshold measure is the frequency that maintains 50% of the maximal response rate (Miliaressis et al. 1986). This approach of rate-frequency analysis has been used in this study.

Effects of drugs on reward can be studied using the electrical self-stimulation paradigm. The mesolimbic dopamine system consists of dopamine neurons with cell bodies in the VTA projecting to the NAS. Previous studies have suggested that the mesolimbic dopamine system is involved in the rewarding and addictive effects of drugs of abuse such as amphetamine and cocaine (White 1996; Koob and LeMoal 1997). In animal studies, most of drugs abused in humans, such as amphetamine, morphine, cocaine, heroin and nicotine, have been reported to increase electrical brain self-stimulation (Liebman and Cooper 1989; Wise 1996a; Rolls 1999).

Excitatory amino acid glutamate may also play an important role in electrical self-stimulation. Systemic administration of the non-competitive NMDA receptor antagonists PCP and MK-801 decreased reward thresholds (Corbett, 1989; Herberg and Rose, 1989). Administration of PCP, MK-801 and CPP into the NAS shell also decreased reward thresholds (Carlezon and Wise, 1996a). Although these studies have reported the effects of NMDA receptor antagonists on electrical self-stimulation, little is known about the effects of AMPA receptor-related compounds. Therefore, the effect of AMPA/kainate receptor antagonist on electrical self-stimulation was investigated in the present study.

1.4.3. *In Vivo* Microdialysis

In vivo microdialysis is a brain perfusion technique which utilizes the concept of an artificial blood vessel surgically inserted into the brain tissue (Ungerstedt 1991). Neurotransmitters in the extracellular space of the brain can be analyzed using *in vivo* microdialysis and high pressure liquid chromatography (HPLC). There are several advantages using *in vivo* microdialysis. Neurotransmitters released into the synapse can be analyzed in freely moving and conscious animals. This allows an evaluation of the effect of drugs on behavioural and neurochemical changes in the brain simultaneously. Microdialysis samples can be collected for a relatively long period of time with less damage to the tissue. These samples collected through the dialysis membrane are relatively free of contamination caused by large molecules, such as proteins and enzymes. Therefore samples can be analyzed using a HPLC system without a preliminary purification step (see Nakahara et al. 1993; Adell and Artigas 1998). A schematic diagram of *in vivo* microdialysis and HPLC is presented in Figure 1.4.

A classical criterion for neurotransmitter release is its sensitivity to the sodium channel blocker tetrodotoxin (TTX). Neurotransmitter release from the synaptic terminal is blocked by infusion of TTX during microdialysis. However, TTX dependency varies with each neurotransmitter in microdialysis. For example, dopamine and acetylcholine release are more than 95% dependent whereas 5-HT and noradrenaline release are approximately 80% dependent on functional sodium channels (Westerink 1995). However, release of the amino acid neurotransmitters glutamate and γ-aminobutyric acid (GABA) appears to be independent of TTX blockade (Timmerman and Westerink 1997). These effects may be due to high affinity uptake systems in the neuronal and glial cells

for these amino acid neurotransmitters. Therefore, a glutamate uptake inhibitor was used to study the role of elevated extracellular glutamate levels in the NAS on neurochemistry and behaviour in this study.

1.5. Thesis Objectives

Hypofunction of NMDA receptors has been proposed in the pathophysiology of schizophrenia (Krystal et al. 1994; Bunney et al. 1995; Olney and Farber 1995). AMPA/kainate receptors may also contribute to glutamate involvement in schizophrenia. The AMPA/kainate receptor antagonists may have differential effects on behaviour relative to the NMDA receptor antagonists (Hauber and Andersen 1993; Svensson et al. 1995; Bubser et al. 1995; Svensson 2000). Furthermore, interactions between glutamate and dopamine may also depend on the relative involvement of D1-like and D2-like dopamine receptors (Martin et al. 1994; Carlsson et al. 1997).

The objectives of this thesis were:

1) To investigate the effect of the glutamate uptake inhibitor PDC on extracellular glutamate and dopamine levels and on locomotor activity.
2) To investigate the effect of the glycine/NMDA receptor agonist glycine on electrical brain self-stimulation and on amino acid levels in the brain, liver and plasma.
3) To investigate the effect of the AMPA/kainate receptor antagonists CNQX and NBQX with D2-like dopamine agonists on locomotor activity.
4) To investigate the effect of CNQX with a D1-like dopamine agonist on locomotor activity.
5) To investigate the effect of CNQX and a D2-like dopamine agonist on electrical brain self-stimulation.

Figure 1.1. Major afferent pathways to the NAS.

AMY, amygdala; HC, hippocampus; NAS, nucleus accumbens; PFC, prefrontal cortex; RN, raphé nucleus; SN, substantia nigra; TH, thalamus; VTA, ventral tegmental area. (Adapted from Bardo 1998; Ikemoto and Panksepp 1999)

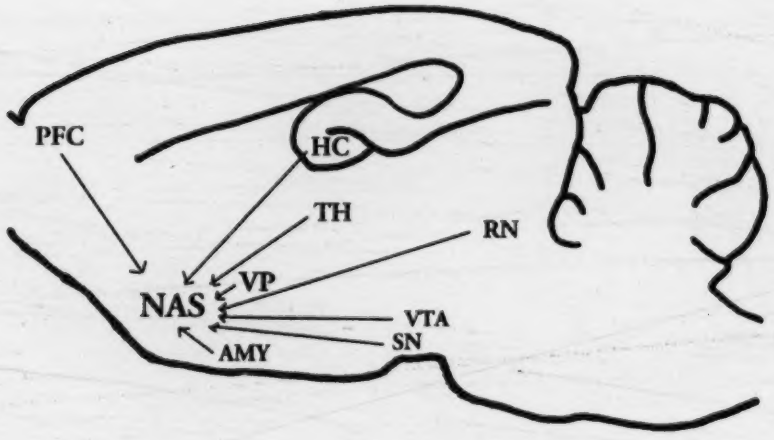


Figure 1.2. Major efferent pathways from the NAS.

AMY, amygdala; BST, bed nucleus of stria terminalis; HC, hippocampus; LH, lateral hypothalamus; LPO, lateral preoptic area; NAS, nucleus accumbens; PAG, periaquaductal grey; PFC, prefrontal cortex; PPN, pedunculopontine nucleus; RN, raphé nucleus; SN, substantia nigra; TH, thalamus; VP, ventral pallidum; VTA, ventral tegmental area. (Adapted from Bardo 1998; Ikemoto and Panksepp 1999)

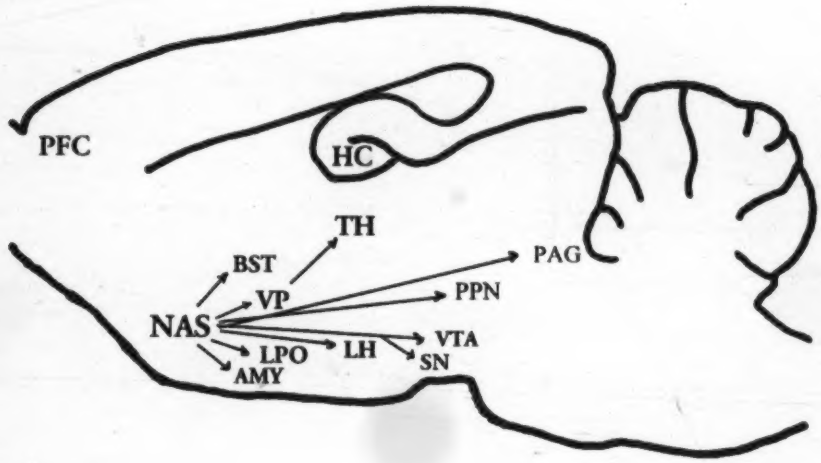


Figure 1.3. Differential inputs and outputs of the NAS core and shell

Aid, dorsal agranular insular cortex; AIv, ventral agranular insular cortex; BAC, basal amygdaloid complex; CA1, cornu Ammonis field 1; CM, central medial nucleus; IL, infralimbic cortex; IMD, intermediodorsal nucleus; PLd, dorsal prelimbic cortex; PLv, ventral prelimbic cortex; PVa, anterior paraventricular nucleus; PVp, posterior paraventricular nucleus; SNC, substantia nigra pars compacta; SNR, substantia nigra pars reticulata; Sub, subiculum; VPdl, dorsolateral ventral pallidum; VPvl, ventrolateral ventral pallidum; VPvm, ventromedial ventral pallidum; VTA, ventral tegmental area

(Adapted from Groenewegen et al. 1999)

Table 1. Characteristics of dopamine receptors

Class	Subtypes	Structure	Biochemical Response	Agonists	Antagonists
D1-like	D1	G protein-coupled (α_s) MW 72,000	↑ AC ↑ PLC ? ↓ Na^+/H^+ exchange ? ↑ K^+ current ?	SKF 38393	SCH 23390
	D5	G protein-coupled (α_s)	↑ AC ↑ IP_3/DAG	SKF 38393	SCH 23390
D2-like	D2 (short) D2 (long)	G protein-coupled (α_i) MW 85,000-150,000	↓AC ↑ K^+ current ↓ Ca^{2+} current ↑ PLC ? ↑ AA ?	Quinpirole PHNO	Sulpiride Haloperidol
	D3	G protein-coupled (α_i)	↓ AC	7OH-DPAT 7OH-PIPAT Quinelorane	UH 232
	D4	G protein-coupled (α_i)	↓ AC		Clozapine Olanzapine

AC, Adenylyl cyclase; PLC, Phospholiphase C; IP_3, Inositol triphosphate; DAG, Diacyl glycerol; AA, Arachidonic acid

(Adapted from Strange 1993, Seeman 1995, Neve and Neve 1997)

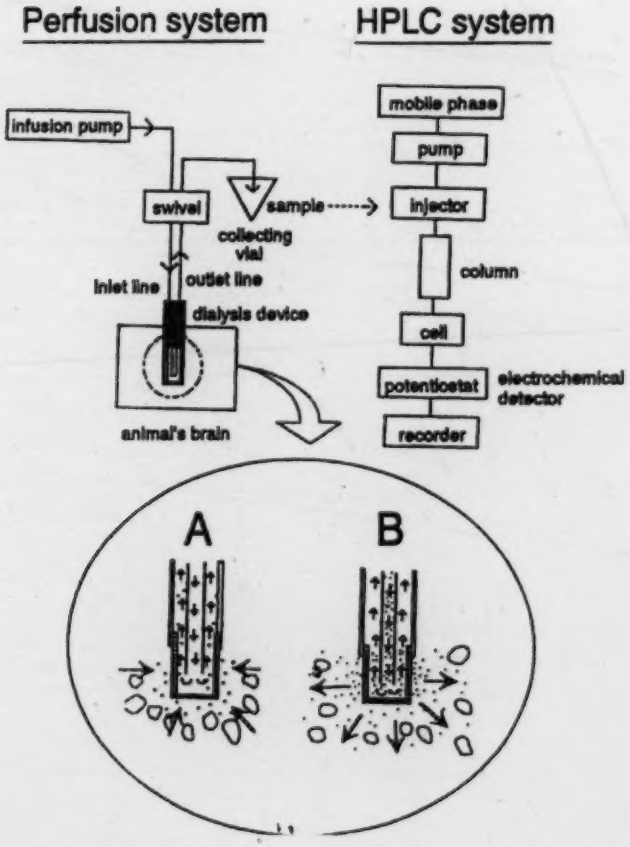
Table 2. Characteristics of ionotropic glutamate receptors

Subfamily	Subunit	Channel Assembly	Characteristics	Agonists	Antagonists
NMDA receptor	NMDAR1	Homomeric or Heteromeric	Seven splice variants NMDAR1A-G	NMDA Glycine	D-AP5 D-CPP PCP MK-801
	NMDAR2	Heteromeric	Four splice variants NMDAR2A-D		
AMPA receptor	GluR1 GluR2 GluR3 GluR4	Homomeric or Heteromeric	Flip or flop variants RNA splicing GluR2 limits Ca^{2+} permeability	AMPA	CNQX DNQX NBQX
Kainate receptor	GluR5 GluR6	Homomeric or Heteromeric	Low affinity KA binding	Kainate Domoic acid	CNQX DNQX
	GluR7 KA1 KA2	Heteromeric	High affinity KA binding		

KA, kainic acid.

(Adapted from Bettler and Mulle 1995; Cotman et al. 1995, Dingledine et al. 1999)

Figure 1.4. Schematic drawing of *in vivo* microdialysis and HPLC system. A) Extraction of endogenous compounds from the extracellular space of the brain. B) Introduction of chemical substances into the brain extracellular space (Adapted from Nakahara et al. 1993).



CHAPTER 2. MATERIALS AND METHODS

2.1. Drugs

Drugs used in this study are L-*trans*-pyrrolidine-2,4-dicarboxylic acid (PDC), glycine, 6-cyano-7-nitro-1,2,3,4-tetrahydro-quinoxaline-2,3-dione (CNQX), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide (NBQX), (+)-amphetamine, (±)-2-dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene (7-OH-DPAT), *trans*-(-)-4aR-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1H-pyrazolo[3,4-g]quinoline [(-)-quinpirole], (+)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol [(+)-SKF 38393]. PDC, CNQX, NBQX, 7-OH-DPAT, (-)-quinpirole and (+)-SKF 38393 were purchased from RBI. Glycine was purchased from Sigma and (+)-amphetamine was from SmithKline Beecham Pharma. Structures of drugs are represented in Figures 2.1-2.4.

2.2. Stereotaxic Surgery

2.2.1. Subjects

Male Sprague-Dawley rats (200-250 g) were obtained from Health Sciences Laboratory Animal Services, University of Alberta. The animals were individually housed in Plexiglas cages on wooden chip bedding in a temperature (21±1 °C) and humidity-controlled environment with a 12-h light/dark cycle (7:00-19:00 h). Food and water were freely available in the home cages. The standard animal feed (Lab-Blox feed, Wayne Feed Division, Continental Grain Company, Chicago, IL, USA) was composed of

4.0% crude fat, 4.5% crude fiber and 24% crude protein. The care and use of animals in the study conformed to the current guidelines of the University of Alberta Health Sciences Animal Welfare Committee and the Canadian Council on Animal Care.

2.2.2. Stereotaxic Surgery

The stereotaxic coordinates for the NAS were [mm]: anterior-posterior (AP) +10.6, medial-lateral (ML) ±1.2, dorsal-ventral (DV) +5.2; core AP +10.1, ML ±2.0, DV +4.2; shell AP +10.1, ML ±0.2, DV +3.7 from interaural zero with the incisor bar set at 2.4 mm below interaural zero according to the atlas of Paxinos and Watson (1986). These coordinates were interpolated from the target site for an angle of 8° (NAS central and core) and 16° (NAS shell) from the sagittal plane to minimize damage to the cerebroventricular system (Greenshaw 1997).

Animals were anaesthetized using sodium pentobarbital (60 mg/kg, i.p.) and placed in a Kopf stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). A midline incision was made, and the skull scraped clean and the bregma exposed. The points of entry into the skull for bilateral guide cannulae were determined and the holes were drilled with a Dremel moto-tool (Racine, WI, USA). Four additional screw holes were drilled for the stainless steel screws (Lomat Watch Co. Montréal, PQ, Canada) used to secure the cannulae and dental acrylic. Stainless steel guide cannulae (22 gauge, Plastics One, Roanoke, VA, USA) were slowly lowered into the target sites bilaterally and secured with four stainless steel screws and dental acrylic. Stainless steel stylets were placed inside the guide cannulae to prevent occlusion. The guide cannulae were

implanted such that their tips were 1.0 mm away from the actual microinjection sites. The wound was closed with two stainless steel wound clips. The animal was removed from the stereotaxic frame, antibiotic eye wound powder was applied (G&E Pharmacy, Edmonton, AB, Canada), and the animal was kept warm under light until full recovery from anesthesia. All surgical procedures were performed under aseptic conditions using antibiotic detergent solution (Savlon, Zeneca Pharma, Mississauga, ON, Canada), 70% ethanol and 0.9% sterilized saline. The animals were allowed to recover from surgery for at least one week before behavioural testing.

2.3. Intracranial Microinjection and Locomotor Activity

2.3.1. Photobeam Activity Apparatus

Spontaneous locomotor activity was monitored using six computer-monitored photobeam boxes (I. Halvorsen System Design. Phoenix AZ, USA) consisting of Plexiglas test cages (43×43×30 cm) each placed in a 12×12 beam infrared grid system 2.5 cm above the floor. Vertical activity was measured with additional 12 beams at a height of 12 cm above the floor. Total activity, consecutive activity and vertical activity were monitored using this computer-monitored photobeam activity system.

2.3.2. Preparation of Drug Solutions

Vehicle and drug solutions were prepared on the day of microinjection. Drugs were dissolved in 0.9% isotonic saline or 45% 2-hydroxy-β-cyclodextrin (HBC) solution.

The CNQX:HBC complex was dissolved in 0.45% saline to obtain 1 μg/μl CNQX. The range of pH values of the drug solutions was between 5.0 and 7.0. Artificial cerebrospinal fluid (aCSF), pH 7.2 or 45% HBC solution was used as vehicle solution. The artificial CSF (NaCl 147 mM, KCl 4.0 mM, $CaCl_2$ 2.3 mM, $MgCl_2$ 1.0 mM, pH 7.2) was prepared and filtered with a 0.2 μm membrane filter and degassed under vacuum before use.

2.3.3. Microinjection and Locomotor Activity

One week after surgery, animals were handled and habituated to the test environment for three days. Each animal was picked up and wrapped in a small towel and gently held with its head exposed for a couple of min. Following this handling procedure, each animal was placed in a photobeam activity box for 60 min to habituate to the test environment.

Following the habituation period, animals received intracranial microinjections according to a repeated-measures design. Unless otherwise specified, each animal received four microinjection and test periods with three days between each microinjection, according to a Latin square design. For the microinjection procedure, the stylets were removed from the guide cannulae and microinjection cannulae (26 gauge, Plastics One, Roanoke, VA, USA) were slowly lowered into the target sites. Microinjection cannulae were 1 mm longer than the tips of guide cannulae in the NAS core or shell. Bilateral microinjections (0.5 μl/side) were delivered into the NAS core or shell over 2.5 min using polyethylene (PE) 10 tubing (Fisher Scientific Co. Pittsburgh, PA, USA) and 10 μl microsyringes (Hewlett Packard Co.) mounted on a syringe drive

attached to a Beehive Controller (BAS Inc. West Lafayette, IN, USA). A small air bubble was introduced between the drug solution and distilled water in the PE 10 tubing to verify the volume of drug solution injected. After the microinjection, injection cannulae remained in place for 1 min to allow the drug solution to be absorbed into the surrounding tissue. The microinjection cannulae were slowly removed and stylets replaced into the guide cannulae. Following microinjection (approximately 5 min), each animal was placed in the photobeam activity box and spontaneous locomotor activity was monitored for 60 min.

The following measurements were taken:

- *Total Activity: total number of beam breaks, indicating all locomotor behaviour*
- *Vertical Activity: number of upper beam breaks, indicating rearing behaviour*
- *Consecutive Activity: repetitive breaking of same beam, indicating stereotyped behaviour*

2.4. Intracranial Self-stimulation (ICSS)

2.4.1. Electrode and Cannulae Implantation

Surgery was carried out under sodium pentobarbital anesthesia (60 mg/kg, i.p.) under aseptic conditions. Each animal was implanted, using a Kopf stereotaxic instrument (Kopf Instruments, Tujunga, CA, USA), with a monopolar nichrome electrode (200 μm diameter; Plastics One, Roanoke, VA, USA) directed to the VTA, and bilateral guide cannulae (22 gauge, Plastics One, Roanoke, VA, USA) into the NAS core or shell. A

large silver indifferent electrode was placed on the skull. Stereotaxic coordinates for the VTA and the NAS were [mm]: VTA AP +3.2, ML +0.6, DV +2.3; NAS core AP +10.1, ML ±2.0, DV +4.2; and NAS shell AP +10.1, ML ±0.2, DV +3.7 from interaural zero according to the atlas of Paxinos and Watson (1986). The coordinates were interpolated from the target site for an angle of 20° from the sagittal plane and 20° from the coronal plane for the VTA, 8° for the NAS core and 16° for the NAS shell from the sagittal plane (Greenshaw 1997). The guide cannulae were implanted 1 mm away from the actual injection sites. Cannula and electrode placements were verified at the end of the experiment by microscopic inspection of frozen coronal sections (40 μm) of the brain.

2.4.2. ICSS Apparatus

The experiments were carried out in six operant test chambers (24×30×29 cm) each equipped with a sound-attenuating outer chamber (Coulbourn Instruments Ltd, Lehigh Valley, PA, USA). Gold track slip rings were used to connect the animals to constant current programmable stimulators (I. Halvorsen Systems Design. Brownwood, TX, USA). With these devices, stimulus current, frequency and pulse width were under computer control. The control of experiments and recording of behavioural responses were achieved with a microcomputer (IBM 486 PC).

2.4.3. ICSS Procedure

Beginning 1 week after surgery, each animal was trained to respond on the lever, resulting in delivery of the VTA stimulation consisting of 1-sec trains of cathodal 0.2-

msec pulses at 100 Hz (6 days a week). Training sessions were of 1 hr duration and were conducted for at least 7 days. The rate of responding was then determined at a function of current intensity. The current intensity that maintained half-maximal rates of responding was determined from a linear regression analysis of the relationship between response rate and current intensity at 10 μA steps for at least three days. Following a "method of limits" procedure (Gallistel and Karras 1984), the animals were then tested daily under conditions whereby the number of pulses per train was systematically varied, initially at the current determined above. Sessions began with animals responding for trains of 160 Hz. At 60-sec intervals, the frequency was decreased in 0.1 log steps until the animal stopped responding, then increased in an equivalent manner until 160 Hz trains were again delivered. At the start of each 60-sec interval, each animal received three trains of stimulation (primes) at the frequency that was available during each period. This priming stimulation served as a discriminative stimulus to indicate the stimulus characteristics. With this feature of the schedule *ad hoc* experimenter-delivered stimulation was never given at the beginning of the ascending frequency steps. The total number of responses in each 60-sec interval was recorded. With this schedule the session length varied between 2 and 26 min. If the animal did not respond, then the session was terminated after repeating the first frequency (160 Hz) bin; if the animal responded at each of the 13 frequencies (160-10 Hz), then each frequency bin was run twice, yielding a maximal session length of 26 min. After initial training of 5-7 days, current was adjusted for each animal so that the half-maximal response rate occurred at around the middle of the range of frequencies. Sessions then continued for at least 5 days until responding had stabilized.

The following measurements were taken:

- *M50: the frequency that maintained half-maximal response rates*
- *RMAX: the maximal number of responses at a single frequency*
- *TRES: the total number of responses per session*
- *ZFRE: the frequency at which response rates dropped to zero*

2.5. *In vivo* Microdialysis and HPLC Assay

2.5.1. Cannula Implantation

Each animal was anaesthetised with sodium pentobarbital (60 mg/kg, i.p.) and implanted with a concentric type guide cannula (MD-2250, BAS Inc. West Lafayette, IN, USA) in the NAS. The tip of the guide cannula was located 2 mm above the actual site of microdialysis to minimise tissue damage induced by the guide cannula implantation. Each animal was housed individually in a Plexiglas cage and was given 24-48 hr of recovery period prior to the microdialysis experiment. The animals were maintained on a 12 hr light-dark cycle and had free access to food and water in the home cages.

2.5.2. *In Vivo* Microdialysis

In vivo microdialysis experiments were carried out between 8:00 and 18:00 h. On the day of microdialysis, fresh aCSF (NaCl 147 mM, KCl 4.0 mM, $CaCl_2$ 2.3 mM, $MgCl_2$ 1.0 mM, pH 7.2) was filtered using a 0.2 μm membrane filter and degassed under vacuum. Drugs were dissolved in aCSF and adjusted to pH 7.2 with HCl or NaOH. A

concentric type of microdialysis probe (2 mm membrane length, MD-2200, BAS Inc. West Lafayette, IN, USA) was soaked in 70% ethanol for 5 min to remove glycerol on the membrane. The probe was transferred to distilled water and perfused with distilled water for 30 min at a rate of 5 µl/min. The probe was then perfused with aCSF (pH 7.2) at a rate of 2.5 µl/min using a 1 ml microdialysis syringe (BAS Inc. West Lafayette, IN, USA) attached to a Beehive Controller (BAS Inc. West Lafayette, IN, USA). The animal was held gently and the dialysis probe was inserted into the NAS through the guide cannula. The animal was then placed in a microdialysis chamber. The dialysis probe was connected to a two-channel liquid swivel (Instech, Plymouth Meeting, PA, USA) through a teflon tubing and tubing connector (BAS Inc. West Lafayette, IN, USA), allowing free movement of the animal inside the dialysis chamber. Dialysates collected during the first 2 h were discarded to allow the levels of extracellular amino acids and dopamine to stabilise. Two hours after the probe insertion, dialysates were collected every 10 min in microfuge tubes containing 5 µl of 0.1 N HCl and maintained at 4 °C. Samples frozen immediately on dry ice and stored at -70°C until HPLC analysis. After collection of six baseline samples, drug dissolved in aCSF (pH 7.2) was infused continuously into the NAS through the dialysis probe and sampling was continued. With this reverse microdialysis procedure, simultaneous infusion of drug and collection of aCSF in the NAS was performed without any disturbance to the freely moving animals.

2.5.3. HPLC Assay of Dopamine and its Metabolites

Levels of dopamine and acid metabolites in dialysates were analyzed using

reversed phase high pressure liquid chromatography (HPLC) with electrochemical detection. The mobile phase consisted of 55 mM NaH_2PO_4, 0.85 mM sodium octyl sulfate, 0.37 mM EDTA and 10% v/v acetonitrile; the pH was adjusted to 2.9 with phosphoric acid. The mobile phase was filtered with a 0.25 μm membrane filter, degassed under vacuum and pumped into the HPLC system at a flow rate of 0.3 ml/min. Dialysates (30 μl each) were placed in the autosampler at 4 °C and 20 μl was injected on column. Separations were achieved using a Waters Symmetry Shield RP8 column (2.1×150 mm, 5 μm particle size) with the temperature set at 30 °C and protected by a Waters Symmetry Guard C18 column. Dopamine and acid metabolites (DOPAC, HVA and 5-HIAA) were detected by a Waters 460 electrochemical detector with a glassy carbon electrode with the applied potential set at 0.85 volt against a silver/silver chloride reference electrode. Mobile phase delivery and sample management were under control of a Waters Alliance 2690 XE system. Maximum sensitivity to dopamine was 0.78 pg on column. A calibration curve consisting of known, varying amounts of the dopamine and metabolites of interest was run in parallel with each assay. Peak heights of dopamine and acid metabolites in the dialysates were compared to those on a calibration curve in order to determine the quantity of each in the dialysates.

2.5.4. HPLC Assay of Amino Acids

Amino acids in the dialysates were analysed using pre-column fluorogenic derivatization in combination with reversed phase HPLC. o-Phthalaldehyde derivatives were prepared by reacting a 5 μl aliquot of the dialysate with 5 μl of fluoraldehyde

reagent containing o-phthalaldehyde and mercaptoethanol (Pierce chemicals, Rockford, IL, USA). o-Phthalaldehyde reacts with primary amines in an alkaline medium in the presence of the reducing agent mercaptoethanol to form highly fluorescent thioalkyl-substituted isoindoles. The gradient mobile phase consisted of mobile phase A: 80 mM NaH_2PO_4, methanol, acetonitrile and tetrahydrofuran (90:24:2:1 by volume) and mobile phase B: 40 mM NaH_2PO_4, methanol and tetrahydrofuran (134:111:6 by volume). The pH was adjusted to 6.2 using 10 N NaOH, and the solution was filtered through a 0.25 μm filter and degassed under vacuum. Mobile phase A (45%) and mobile phase B (55%) were pumped at a flow rate of 0.3 ml/min initially then changed to mobile phase B (100%) at 15 min. The apparatus for HPLC detection consisted of a Waters Alliance 2690 XE system coupled to an automatic injector. Separations were achieved using a 250 × 4.6 mm analytical column packed with spherical 5 μm particles (Spherisorb ODS2; Phenomenex) and protected by a C18 Bondapak guard column. The derivatives of the amino acids were detected by using a Shimadzu model RF-10A fluorescence detector (Xenon lamp) with an excitation wavelength of 260 nm and an emission wavelength of 455 nm. Peak heights of the amino acids were calculated using an on-line microcomputer (Digital Venturis FX computer with Waters Millennium Software). A calibration curve was run in parallel with each assay using known, varying amounts of the amino acids of interest. Peak heights of amino acids in the dialysates were compared to those on a calibration curve in order to determine the quantity of each amino acid in the dialysates.

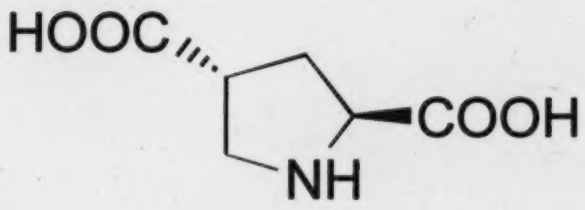
2.6. Histology

After the completion of each set of experiments, animals were deeply anaesthetized with sodium pentobarbital (100 mg/kg, i.p.) and perfused through the heart with ice-cold 0.9% saline followed by ice-cold 10% phosphate buffered formalin solution. Brains were removed and stored in 10% formalin in 30% sucrose solution for at least two weeks. Brains were then sectioned coronally at 40 μm using a cryostat and stained with cresyl violet. Microinjection sites in the NAS core or shell, microdialysis probe sites in the NAS and electrode placement in the VTA were verified under microscopic examination. Histological verification of microinjection sites in the NAS core and shell are represented in Figure 10.7 and 10.8. The placements of electrodes in the VTA are represented in Figure 13.8.

2.7. Statistics

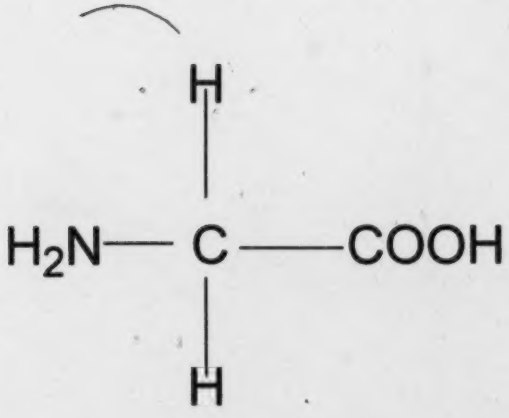
The spontaneous locomotor activity data and *in vivo* microdialysis data were subjected to Analysis of Variance (ANOVA) with a criterion of $P<0.05$ using SPSS 7.5 (SPSS Inc. Chicago, IL, USA) and GraphPad Prism 3.0 (San Diego, CA, USA) software packages. For comparisons between means, following a significant F test, post-hoc multiple comparisons tests (Tukey's or Dunnett's test) were used with a criterion of $P<0.05$. Intracranial self-stimulation data were analyzed by Friedman's test followed by Wilcoxon's matched-pairs signed ranks test. Chronic oral glycine data were analyzed with the Mann-Whitney U test and Wilcoxon's matched-pairs signed ranks test (Siegel 1956; Winer 1971; Keppel 1991; Motulsky 1995).

Figure 2.1. Structures of PDC and of glycine.



L-*trans*-Pyrrolidine-2,4-dicarboxylic acid (PDC)

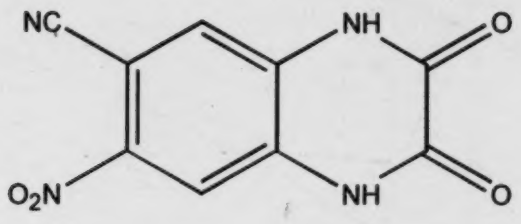
Glutamate uptake inhibitor



Glycine

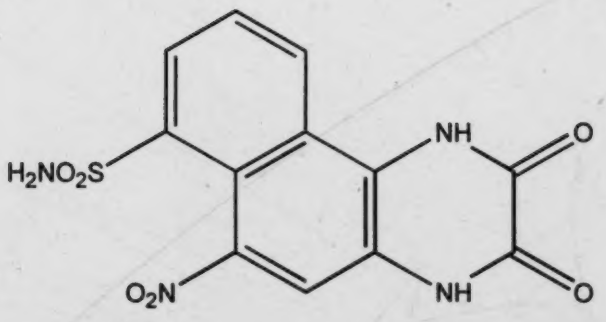
NMDA/glycine receptor agonist

Figure 2.2. Structures of CNQX and of NBQX.



6-Cyano-7-nitro-1,2,3,4-tetrahydro-quinoxaline-2,3-dione (CNQX)

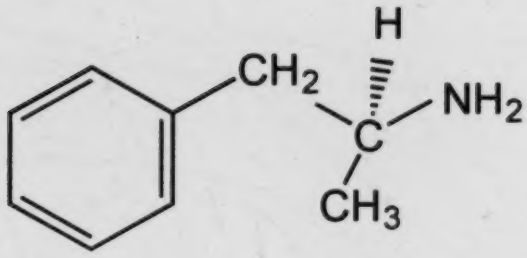
Competitive AMPA/kainate receptor antagonist



2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide (NBQX)

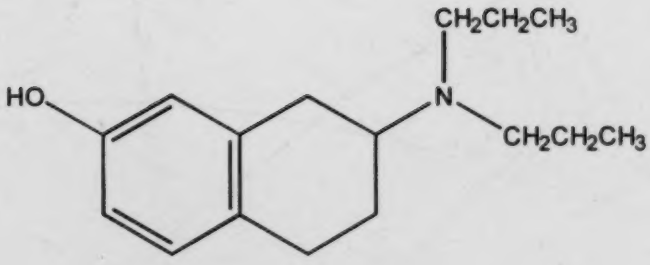
Competitive AMPA/kainate receptor antagonist

Figure 2.3. Structures of (+)-amphetamine and of 7-OH-DPAT.



(+)-Amphetamine

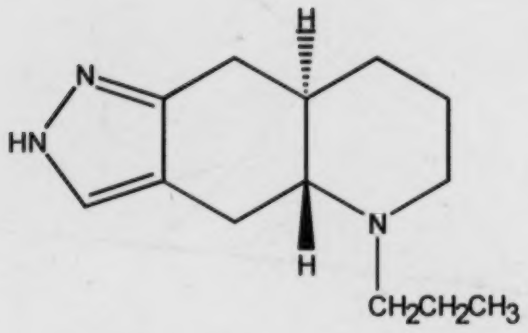
Indirect dopamine receptor agonist



(±)-2-Dipropylamino-7-hydroxy-1,2,3,4-tetrahydronaphthalene [(±)-7-OH-DPAT]

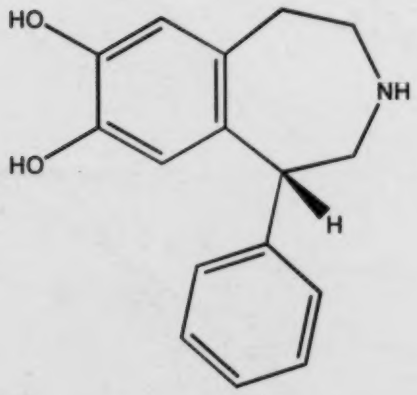
D2/D3 dopamine receptor agonist

Figure 2.4. Structures of (-)-quinpirole and of (+)-SKF 38393.



trans-(-)-4aR-4,4a,5,6,7,8,8a,9-Octahydro-5-propyl-1H-pyrazolo [3,4-g] quinoline

[(-)-quinpirole] D2/D3 dopamine receptor agonist



(+)-1-Phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol [(+)-SKF 38393]

D1 dopamine receptor agonist

3. PDC ELEVATES EXTRACELLULAR GLUTAMATE IN THE NAS: NO EFFECT ON LOCOMOTOR ACTIVITY

3.1. Introduction

L-Glutamate is the major excitatory amino acid neurotransmitter in the CNS. Extracellular L-glutamate concentrations are tightly regulated by excitatory amino acid transporters on neuronal and glial cells to limit glutamate receptor stimulation during neuronal transmission and to prevent excitotoxicity induced by over-stimulation of these receptors (Nicholls 1994; Vandenberg 1998; Seal and Amara 1999). Glutamate uptake inhibitors may be useful pharmacological tools for studying the roles of these transporters involved in the regulation of glutamate transmission at the synapse (Bridges et al. 1999). Based on the NMDA receptor hypofunction hypothesis of schizophrenia (Olney and Farber 1995), it is possible that enhanced glutamate function by a transport inhibitor may have beneficial effects on schizophrenic symptoms. The effects of a glutamate uptake inhibitor on neurochemistry and behaviour were investigated in this experiment.

The glutamate uptake inhibitor L-*trans*-pyrrolidine-2,4-dicarboxylic acid (PDC) has strong excitatory amino acid transporter affinity and negligible glutamate receptor affinity. PDC inhibits sodium-dependent uptake of glutamate into synaptosomes (IC_{50} of 1-5 μM) and the uptake of glutamate into glial cells (IC_{50} of 50 μM) (Bridges et al. 1991). It has been reported that PDC may not induce significant neurotoxicity *in vivo*, in contrast to other glutamate transport inhibitors such as dihydrokainate and *threo*-β-hydroxy-aspartic acid (McBean and Roberts 1985; Massieu et al. 1995). Therefore, PDC

is a good candidate for studying the effects of elevated endogenous glutamate levels on dopamine release in the NAS and locomotor activity.

L-Glutamate may have facilitatory or inhibitory effects on dopamine function depending on relative concentrations of L-glutamate in the synapse (see Whitton 1997; Morari et al. 1998). The effects of PDC on dopamine release in the striatum (including the NAS) are not consistent in microdialysis studies. For example, a low dose of PDC (0.2 mM), when infused in the striatum, increased extracellular dopamine levels (West and Galloway 1997). PDC (1 mM) decreased extracellular dopamine levels in the NAS (Taber et al. 1996) whereas PDC (4 mM) increased dopamine levels in the striatum (Segovia et al. 1997). A higher dose of PDC (10 mM) did not alter extracellular dopamine levels in the striatum (Semba and Wakuta 1998). These authors suggested that 15- to 30-fold increases in extracellular glutamate levels may not be necessary to stimulate dopamine release in the striatum. Another glutamate uptake inhibitor, *threo*-β-hydroxyaspartic acid (50 mM), when infused into the NAS, increased extracellular glutamate levels but did not alter locomotor activity (Dalia et al. 1998); extracellular dopamine levels were not reported in that study. These studies do not agree on the effects of PDC on dopamine release in the striatum, although elevated glutamate levels were consistently observed in all cases. Moreover, little is known about the effects of PDC on behaviour in intact animals.

The purpose of this experiment was to investigate the effects of the glutamate uptake inhibitor PDC on extracellular glutamate and dopamine levels and spontaneous locomotor activity in rats. Although previous studies reported the effects of PDC (less than 10 mM) on dopamine release in the NAS, higher doses of PDC have not been

investigated. Therefore, in the present experiment, a high concentration of PDC (25 mM) was infused into the NAS for 20 min by reverse microdialysis and extracellular levels of glutamate, dopamine and acid metabolites (DOPAC, HVA and 5-HIAA) were analyzed using HPLC with fluorescence or electrochemical detection. It was expected that PDC would elevate extracellular glutamate levels and might alter dopamine release in the NAS. In another experiment, different doses of PDC (0.2, 2 and 4 μg) were administered directly into the NAS and spontaneous locomotor activity was monitored for 60 min. It was hypothesized that elevated extracellular glutamate levels by different doses of PDC may alter basal locomotor activity in rats.

3.2. Methods

3.2.1. *In Vivo* Microdialysis and HPLC Assay

Male Sprague-Dawley rats (250-300 g, n=4) were implanted with a microdialysis guide cannula (MD-2250, BAS Inc. West Lafayette, IN, USA) into the NAS under sodium pentobarbital anesthesia (60 mg/kg, i.p.). Animals were given 24-48 hr of recovery before microdialysis experiments. On the day of microdialysis, a concentric type microdialysis probe with a 2 mm membrane length (MD 2200, BAS Inc. West Lafayette, IN, USA) was inserted into the NAS and infused with artificial CSF (pH 7.2) at a rate of 2.5 μl/min. Dialysates from the first 2 hr were discarded to allow for stabilization of baseline levels of extracellular neurotransmitters. Following collection of six baseline samples (10-min each), PDC (25 mM) dissolved in artificial CSF (adjusted pH to 7.2 with 5 N NaOH) was infused in the NAS for 20 min and artificial CSF was

infused for a further 100 min. Dialysates were collected in microfuge tubes containing 5 µl of 0.1 N HCl in a ice-filled box during the infusion procedure. Collected samples were numbered, labeled and immediately frozen in dry ice and stored at –70 °C until HPLC analysis. Alternate numbered samples were analyzed for amino acids using HPLC with fluorescence detection and the other for dopamine and its acid metabolites using HPLC with electrochemical detection (see Chapter 2.5. for details).

3.2.2. Microinjection of PDC into the NAS

Male Sprague-Dawley rats (200-250 g, n=24) were implanted with bilateral microinjection guide cannulae directed to the NAS under sodium pentobarbital anesthesia (60 mg/kg, i.p.). One week after surgery, the animals were randomized into four groups (vehicle, PDC 0.2 µg, PDC 2 µg and PDC 4 µg: n=6 per group). Each group received one microinjection of PDC (0.5 µl/side, bilateral) into the NAS and the animals were tested for spontaneous locomotor activity for 60 min (see Chapter 2.3. for details).

3.2.3. Statistics

Microdialysis data were analyzed using one-way ANOVA with repeated measures (time) for each compound followed by a *post-hoc* multiple comparisons test (Dunnett's test). An average of three baseline samples before PDC infusion was used as a control in the Dunnett's test. Locomotor activity data were analyzed using two-way ANOVA (between subjects factor: PDC, within subjects factor: time). To compare means following a significant F test, a *post-hoc* multiple comparisons test (Tukey's test) was used with a two-tailed criterion of $P<0.05$.

3.3. Results

3.3.1. Effects of PDC on Extracellular Glutamate and Dopamine

Infusion of PDC (25 mM) into the NAS for 20 min markedly increased extracellular glutamate [$F(1,8)=16.590$, $P<0.05$] and aspartate [$F(1,8)=9.609$, $P<0.05$] levels (Figure 3.1). This PDC effect was reversible because glutamate and aspartate levels returned to baseline when aCSF was infused again into the NAS. There were small but significant increases in asparagine [$F(1,8)=7.304$, $P<0.05$] and glutamine [$F(1,8)=3.323$, $P<0.05$] levels following infusion of PDC (25 mM) into the NAS. Extracellular serine [$F(1,8)=1.179$, $P>0.05$] and glycine [$F(1,8)=1.219$, $P>0.05$] levels were not altered by PDC (25 mM) infusion.

Infusion of PDC (25 mM) into the NAS also decreased extracellular DOPAC [$F(1,8)=55.941$, $P<0.05$] and 5-HIAA [$F(1,8)=19.929$, $P<0.05$] levels (Figure 3.2). These decreases were still evident after 100 min of PDC infusion. Extracellular dopamine [$F(1,8)=1.327$, $P>0.05$] and HVA [$F(1,8)=0.993$, $P>0.05$] levels were not altered by infusion of this drug into the NAS.

3.3.2. Effects of PDC on Locomotor Activity

Administration of PDC (0.2, 2 and 4 μg) into the NAS did not alter total activity [$F(1,3)=0.039$, $P>0.05$]. There was no significant interaction between PDC and time [$F(11,33)=0.539$, $P>0.05$]. The local time-course data (line graph) showed no significant difference between the control and doses of PDC at each 5 min testing period (Figure 3.3). Administration of PDC (0.2, 2, 4 μg) into the NAS did not alter vertical activity

[F(1,3)=0.731, P>0.05] and there was no significant interaction between PDC and time [F(11,33)=0.673, P>0.05] (Figure 3.4). Administration of PDC (0.2, 2, 4 μg) into the NAS did not alter consecutive activity [F(1,3)=0.430, P>0.05] and there was no significant interaction between PDC and time [F(11,33)=0.522, P>0.05] (Figure 3.5).

3.4. Discussion

Infusion of the glutamate uptake inhibitor PDC (25 mM) into the NAS by reverse microdialysis markedly increased extracellular glutamate and aspartate levels. The effect of PDC was reversible because these amino acids returned to basal levels after PDC infusion in the NAS, indicating efficient excitatory amino acid transport systems *in vivo*. This result is consistent with previous reports of elevated excitatory amino acid levels following administration of glutamate uptake inhibitors (Bloc et al. 1995; Segovia et al. 1997; Dalia et al. 1998). Infusion of PDC (25 mM) into the NAS decreased extracellular levels of the monoamine metabolites DOPAC and 5-HIAA indicating an altered metabolism of dopamine and 5-HT in the NAS. However, baseline dopamine and 5-HT levels were not reliably measured due to the sensitivity-limit of the assay under the conditions of this experiment. Consistent with this result, Segovia et al. (1997) found that PDC (4 mM) increased extracellular glutamate but decreased DOPAC in the rat striatum. Although dialysate glutamate levels are generally accepted as TTX-independent in microdialysis studies (Timmerman and Westerink 1997), it has been reported that elevated glutamate levels by PDC may be dependent on TTX infusion and glutamate receptor blockade in the striatum (Rawls and McInty 1997). Despite the consistent effects

of PDC on excitatory amino acid levels, changes in dopamine levels after PDC infusion were not consistent in previous studies (West and Galloway 1997; Taber et al. 1996; Segovia et al. 1997; Semba and Wakuta 1998). This may be due to different doses of PDC and brain areas used in those studies.

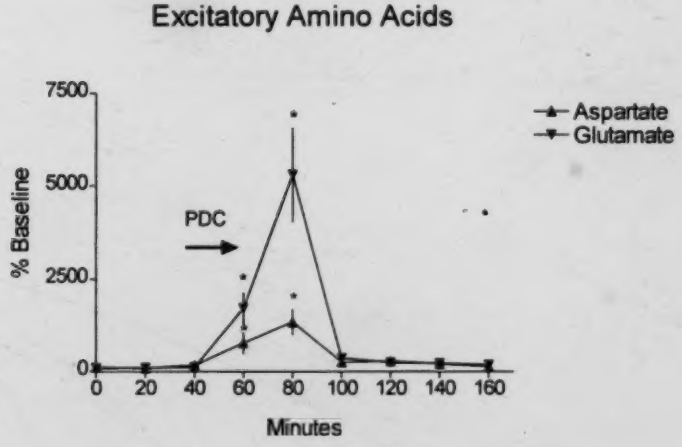
Intra-NAS administration of various doses of PDC (0.2, 2 and 4 μg) did not alter basal locomotor activity in this experiment. Due to possible neurotoxic effects of PDC *in vivo*, PDC was microinjected only once into each animal and tested. The lack of locomotor activity of this glutamate uptake inhibitor is consistent with those from a previous study (Dalia et al. 1998). Another glutamate uptake inhibitor, *threo*-β-hydroxy-aspartic acid (50 mM), when infused into the NAS, had no effect on locomotor activity in rats (Dalia et al. 1998). In contrast, Kim and Vezina (1999) reported that intra-NAS administration of PDC (0.5-10 nmole), doses similar to those used in the present study, increased horizontal and vertical activity in rats. These authors used different microinjection sites in the NAS (anterior vs. posterior) and a different experimental design (within subject vs. between subject) compared to those of the present experiment. Although these results are not consistent, little information is available on the effects of glutamate uptake inhibitors on locomotor behaviour.

It has been suggested that high concentrations of glutamate produce neuronal excitotoxicity *in vitro* (Choi 1988). The excitotoxic effect of glutamate *in vivo* may be dependent on high affinity glutamate uptake systems in neuronal and glial cells. For example, inhibition of glutamate uptake using PDC (100 μM) induced glutamate neurotoxicity in hippocampal neuronal cultures *in vitro* (Robinson et al. 1993). However, *in vivo* administration of PDC (400 nmol) by reverse microdialysis did not induce

excitotoxicity when assessed by alteration of choline acetyltransferase and glutamate decarboxylase levels after PDC infusion (Massieu et al. 1995). The doses of PDC (0.2-4 μg, equivalent to 2.5-50 nmol) used in the present experiment are much lower than the dose (400 nmol) in the study of Massieu et al. Even though it is difficult to compare the results from microdialysis and microinjection studies due to different volume and infusion time used, it is unlikely that the lack of locomotor activity by acute PDC infusion is attributable to excitotoxicity.

Elevated extracellular glutamate levels in the NAS had no significant effect on basal locomotor activity. Stimulation of different subtypes of glutamate receptors, such as ionotropic and metabotropic receptors, has been reported to produce differential effects on behaviour. Therefore, a lack of effect of PDC on locomotor activity may reflect multiple actions of elevated extracellular glutamate on different subtypes of receptors that may oppose each other. Among the glutamate receptors, the NMDA receptors have been implicated in the pathophysiology of schizophrenia. Facilitation of the NMDA receptors using a direct NMDA receptor agonist may cause excitotoxicity by over-stimulating NMDA receptors. Recent studies have suggested that glycine, which is a co-agonist at the NMDA receptor, may have less neurotoxicity than direct NMDA receptor agonists (Leeson and Iversen 1994). Therefore, in the next experiment (Chapter 4), the effects of chronic glycine were investigated on electrical brain self-stimulation and amino acid levels in brain, liver and plasma.

Figure 3.1. Effect of PDC on extracellular amino acid levels in the NAS. PDC (25 mM) was infused into the NAS for 20 min and dialysates were analyzed using HPLC with fluorescence detection. Data are means±S.E.M. *Significant at $P<0.05$, relative to baseline (n=4).



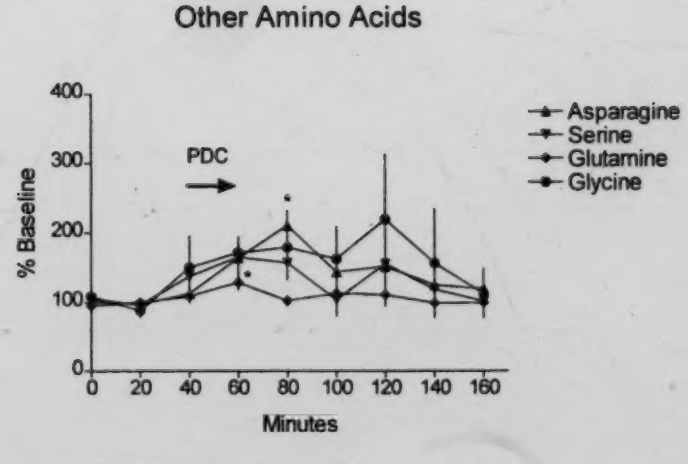


Figure 3.2. Effect of PDC on extracellular dopamine and acid metabolite levels in the NAS. PDC (25 mM) was infused into the NAS for 20 min and dialysates were analyzed using HPLC with electrochemical detection. Data are means±S.E.M. *Significant at $P<0.05$, relative to baseline (n=4).

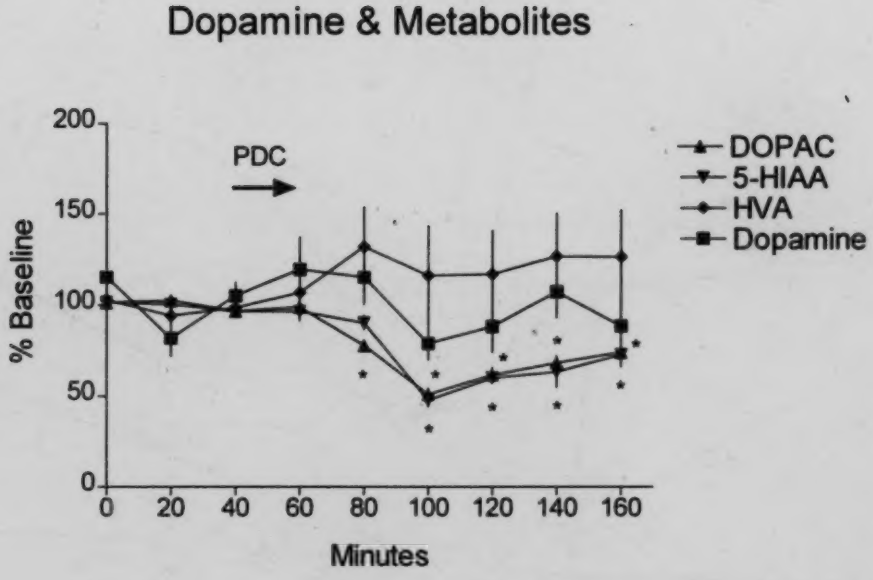


Figure 3.3. Effect of intra-NAS administration of PDC on total activity. PDC (0.2, 2, 4 μg/side, bilateral) was microinjected into the NAS and total activity was monitored for 60 min (n=6). Data are means±S.E.M.

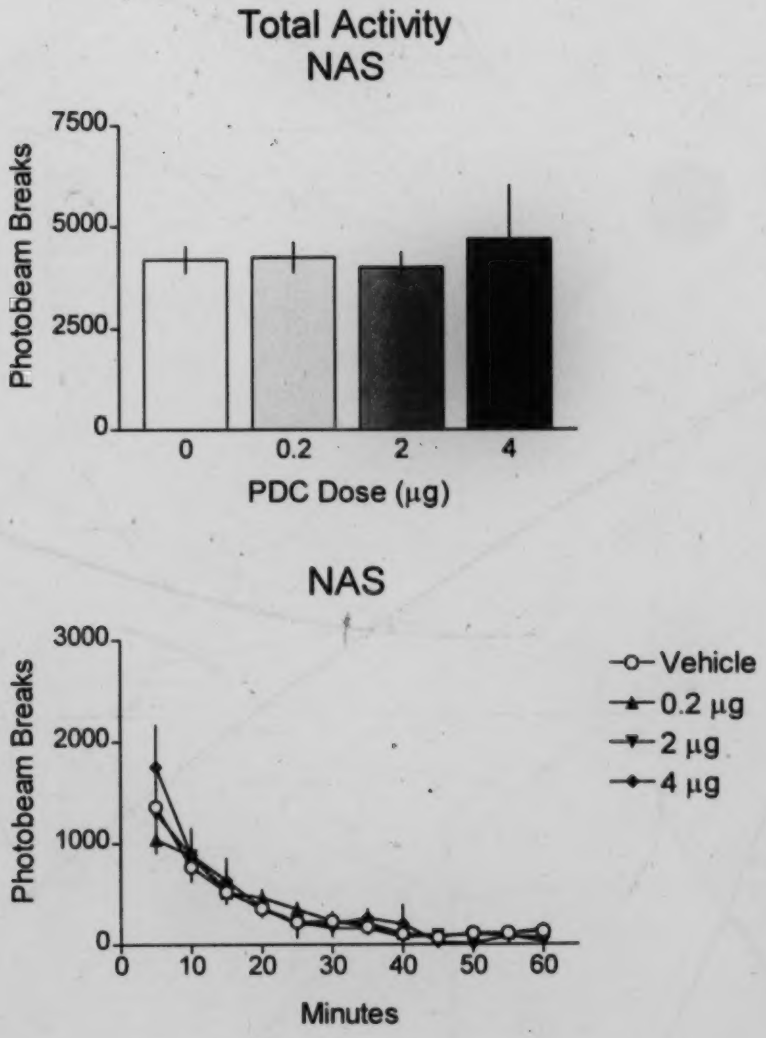


Figure 3.4. Effect of intra-NAS administration of PDC on vertical activity. PDC (0.2, 2, 4 μg/side, bilateral) was microinjected into the NAS and vertical activity was monitored for 60 min (n=6). Data are means±S.E.M.

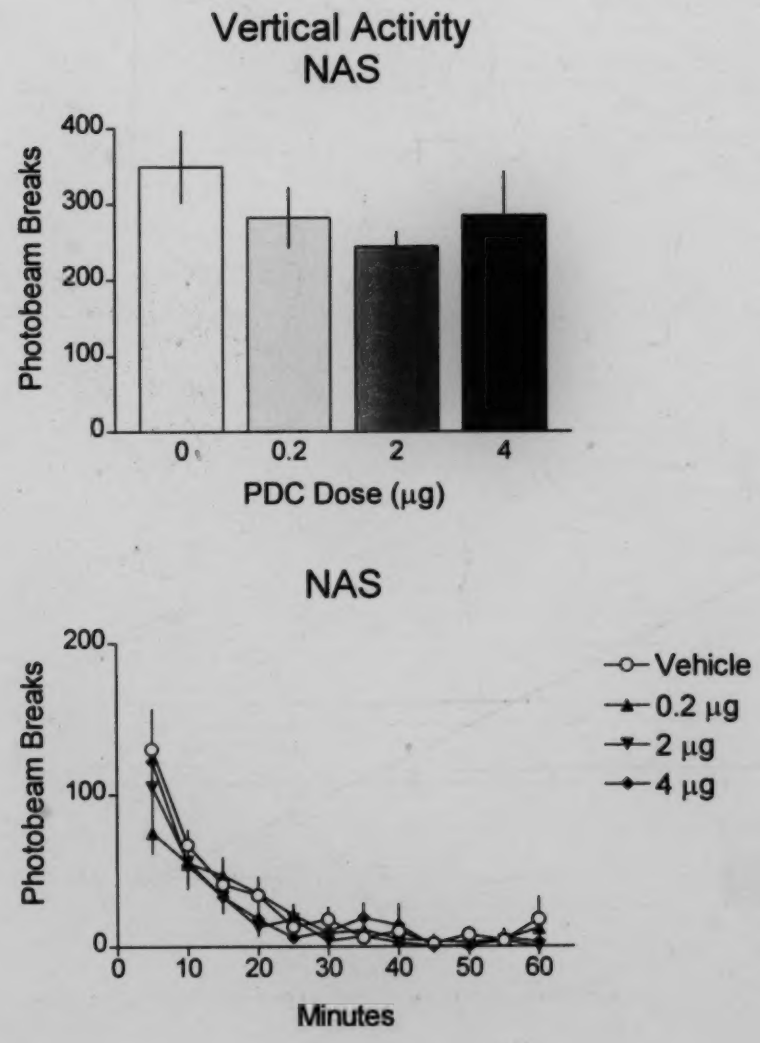
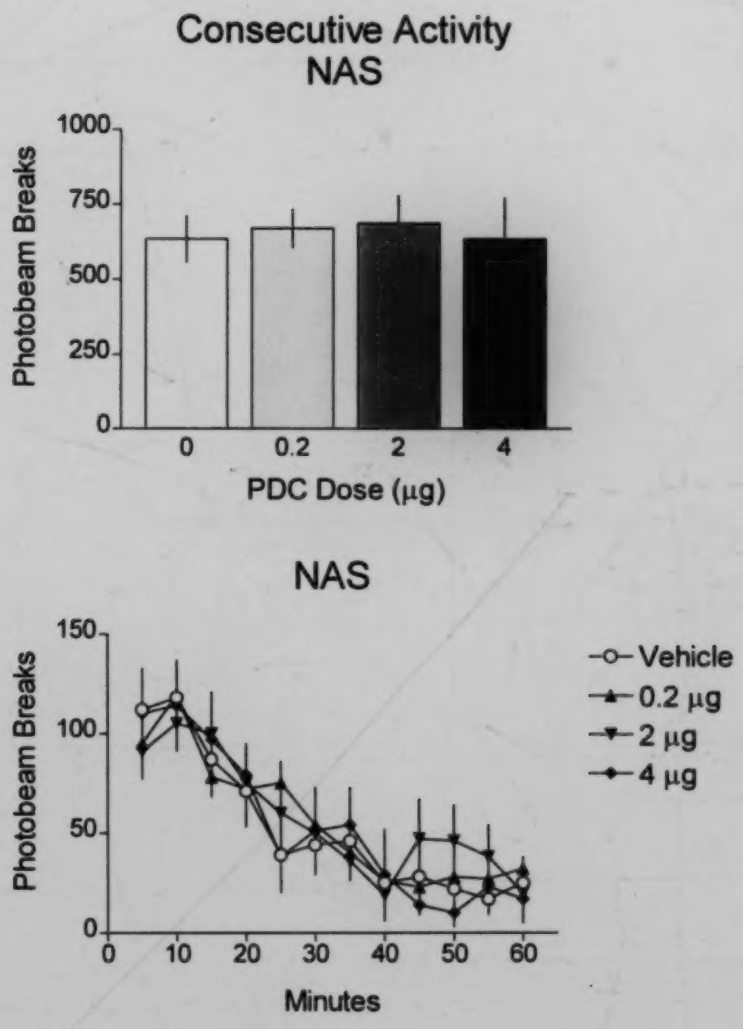


Figure 3.5. Effect of intra-NAS administration of PDC on consecutive activity. PDC (0.2, 2, 4 μg/side, bilateral) was microinjected into the NAS and consecutive activity was monitored for 60 min (n=6). Data are means±S.E.M.



4. CHRONIC ORAL GLYCINE ELEVATES BRAIN GLYCINE LEVELS: NO EFFECT ON BRAIN STIMULATION REWARD

4.1. Introduction

The hypothesis of NMDA receptor hypofunction has been proposed in the pathophysiology of schizophrenia. This hypothesis is based on the observation that the non-competitive NMDA receptor antagonists, phencyclidine (PCP) and ketamine, mimic schizophrenic symptoms in humans (Domino and Luby 1981; Krystal et al. 1994). Administration of PCP to schizophrenic patients has been reported to trigger psychotic symptoms lasting for up to several months (Domino and Luby 1981). Ketamine also induced psychotic symptoms in adults awakening from ketamine anesthesia whereas adolescents showed little susceptibility to this phenomenon (Reich and Silvay 1989). Therefore, ketamine-induced psychosis in adults may have some correlation with peak age onset in schizophrenia. Based on these findings, it was suggested that facilitation of NMDA receptor function may be beneficial as a treatment for schizophrenia (Deutsch et al. 1989; Javitt and Zukin 1991; Farber et al. 1999).

Although facilitation of NMDA receptor function may have clinical efficacy in schizophrenia, direct NMDA receptor agonists may not be good candidates for this treatment due to neurotoxicity caused by over-stimulation of NMDA receptors. The amino acid glycine is an agonist at the strychnine-insensitive glycine binding site of the NMDA receptor (Kleckner and Dingledine 1988; Kemp and Leeson 1993). Both glutamate and glycine sites on the NMDA receptor must be occupied to trigger opening

of this ion channel (Mayer and Westbrook 1987; Thompson 1990). Although glycine is present in the brain at all times, the levels of glycine in the synaptic cleft may not be saturated due to the high capacity of glycine transporters (Wood 1995; Supplisson and Bergman 1997). Therefore, use of glycine has been proposed to mildly augment NMDA receptor function in the brain (Johnson and Ascher 1987).

Some clinical studies demonstrated the improvement of schizophrenic symptoms using agonists at the glycine site of the NMDA receptors. Chronic oral glycine (0.8 g/kg/day, 14 days) administration to schizophrenic patients improved negative symptoms of schizophrenia (Heresco-Levy et al. 1996). D-cycloserine (50 mg/day, 14 days), a partial agonist at the glycine site of NMDA receptor, also improved negative symptoms when given in combination with conventional antipsychotics (Goff et al. 1995). D-serine (30 mg/kg/day, 6 weeks), a full agonist at the glycine site of NMDA receptor, improved schizophrenic symptoms when given with antipsychotics such as sulpiride, haloperidol and risperidone in a double-blind and placebo-controlled trial (Tsai et al. 1998). These clinical studies suggest that glycinergic agonists at the NMDA receptor may have antipsychotic properties when used with other antipsychotic drugs.

Animal studies also provided evidences for the possible utility of glycine therapy in schizophrenia. Toth and Lajtha (1986) reported that administration of a liquid diet containing glycine (50 mg/ml) significantly elevated brain glycine levels in mice. Administration of D-cycloserine (0.375-3 mg/kg, i.p.) inhibited hyperactivity induced by the dopamine releaser methamphetamine and by the D2/3 dopamine receptor agonist quinpirole in rats (Dall'Olio et al. 1994). High doses of glycine (3 g/kg, i.p.) and D-serine (4 g/kg, i.p.) decreased NMDA receptor antagonist MK-801-induced locomotor

hyperactivity in mice (Nilsson et al. 1997). Javitt et al. (1999), using *in vivo* microdialysis, reported that glycine (0.8 g/kg, i.p.) attenuated dopamine release in the NAS and the locomotor hyperactivity induced by PCP in rats. However, despite a number of pieces of evidence suggesting effects of glycinergic agonists on neurochemistry and behaviour in animals, little is known about the effects of these compounds on intracranial self-stimulation (ICSS) in rats. This is somewhat surprising because this experimental paradigm has been widely used to assess potential antipsychotic efficacy in animals.

The purpose of this experiment was to investigate the effects of a clinically relevant dose of glycine (0.8 g/kg/day, 2 weeks, p.o.) on ICSS and several amino acid levels in the brain, liver and plasma of rats. Although Herberg and Rose (1990) reported a lack of effect of acute administration of D-cycloserine (3-15 mg/kg, i.p.) on locomotor activity and ICSS, the effect of chronic oral glycine on ICSS has not been reported. Based on previous clinical studies, it was hypothesized that chronic oral glycine administration may alter ICSS reward threshold in rats. Although a previous study reported elevated brain glycine levels following administration of glycine (Toth and Lajtha, 1986), other amino acid levels in the brain, liver and plasma have not been reported in this context.

4.2. Methods

4.2.1. Chronic Glycine Administration and ICSS

Male Sprague-Dawley rats (250-300 g, n=14) were implanted with a VTA stimulating electrode under sodium pentobarbital anesthesia (60 mg/kg, i.p.). Beginning

one week after surgery, animals were trained to lever-press for delivery of electrical stimulation of the VTA on a continuous reinforcement schedule. Reward thresholds and rate responses were measured as dependent variables: the frequency that maintains half-maximal response rates (M50), the maximal number of responses at a single frequency (RMAX) and the total number of responses in a session (TRES). After training, animals were randomly allocated to groups (vehicle, glycine n=7 per group). Chronic oral glycine (0.8 g/kg/day, 2 weeks, p.o.) or vehicle (0.9% saline) was administered and 30 min later animals were tested for ICSS daily. On the 14th day of glycine administration, amphetamine was injected (0.75 mg/kg, s.c.) 15 min after glycine administration and another 15 min later, ICSS was measured. Glycine administration was continued for another three days to ensure a complete washout of amphetamine. As the brains were used for amino acids analysis, histological verification of stimulation sites was not carried out. However, typical sites for VTA stimulation are illustrated by the data in Figure 13.8. and ICSS was considered to be functional verification in this experiment.

4.2.2. Tissue Sample Preparation

Thirty min after glycine administration, the animals were killed using guillotine decapitation and blood samples were collected in vacu-containers containing EDTA. Brains were rapidly removed, placed immediately in isopentane over solid carbon dioxide, then transferred to vials and stored at –80 °C until HPLC analysis. Liver tissue was rapidly removed and kept frozen at –80 °C. Blood samples were centrifuged with 10,000 g for 10 min at 4 °C, plasma samples were separated out and stored at –80 °C until HPLC analysis.

Half of the whole brain was weighed and homogenized in 5 volumes of ice-cold distilled water. For liver tissue, small pieces were taken from several different areas, weighed and homogenized in 5 volumes of ice-cold distilled water. Homogenate (100 µl) was added to 400 µl of internal standard (homoserine) in ice-cold methanol. This diluted homogenate was quickly re-homogenized with a small pestle in a microfuge tube and centrifuged at 10,000 g for 2 minutes. Plasma samples were quickly homogenized with two volumes of internal standard (homoserine) in ice-cold methanol and centrifuged at 10,000 g for 2 minutes. A 20 µl aliquot of the supernatant was used for the amino acid assay.

4.2.3. HPLC Assay of Amino Acids in Brain, Liver and Plasma

Amino acids in brain, liver and plasma were analyzed using pre-column fluorogenic derivatization in combination with reversed phase HPLC. Standard curves of known concentrations of amino acids were run in parallel to each group of samples. Linear standard curves with correlation coefficients of $r^2>0.99$ were obtained routinely. The peak height ratios of amino acids to internal standard in the samples were compared to those of a standard curve to quantify amino acids in each sample (see Chapter 2.5. for details).

4.2.4. Statistics

Within subject data were analyzed using Friedman's test followed by Wilcoxon's signed-ranks test. Comparisons between the vehicle and glycine group were performed

using the Mann-Whitney U test. Amino acid data were compared between the vehicle and glycine groups using the Mann-Whitney U test.

4.3 Results

4.3.1. Effects of Chronic Oral Glycine on ICSS

Chronic oral glycine administration did not alter the reward threshold (M50) relative to vehicle group (day1-13). Administration of amphetamine (0.75 mg/kg) on day 14 significantly reduced the M50 in both the vehicle [χ_r^2 (13)=32.679, P<0.05] and the glycine group [χ_r^2 (13)=27.437, P<0.05]. However, the effects of amphetamine were not different between the vehicle and glycine groups (P>0.05) (Figure 4.1).

Chronic oral glycine administration did not alter maximal responses (RMAX) relative to the vehicle group (day1-13). Administration of amphetamine (0.75 mg/kg) on day 14 had no significant effect on RMAX in either the vehicle [χ_r^2 (13)=18.426, P>0.05] or the glycine group [χ_r^2 (13)=10.276, P>0.05] (Figure 4.2).

Chronic oral glycine administration did not alter total responses (TRES) relative to the vehicle group (day1-13). Administration of amphetamine (0.75 mg/kg) on day 14 significantly increased TRES in both vehicle [χ_r^2 (13)=36.876, P<0.05] and glycine treated animals [χ_r^2 (13)=25.027, P<0.05]. However, the effects of amphetamine were not different between vehicle and glycine groups (P>0.05) (Figure 4.3).

Rate-frequency curves for the vehicle and the glycine groups are presented in Figure 4.4 together with the respective fitted non-linear regression curves. The upper panel represents averaged rate-frequency curves plotted from the data of day 13 (vehicle

or glycine) and day 14 (vehicle+amphetamine or glycine+amphetamine). The lower panel represents non-linear regression curves calculated from the rate-frequency curves. Both the vehicle+amphetamine and the glycine+amphetamine treatment shifted the rate-frequency curves to the left relative to vehicle group, indicating a decrease in reward threshold (M50).

4.3.2. Effects of Chronic Oral Glycine on Amino Acid Levels

Chronic oral glycine administration significantly increased glycine levels in the brain ($P<0.05$), but only to 120% of the levels in the vehicle group. Levels of other amino acids such as aspartic acid, glutamic acid, asparagine, glutamine, serine and GABA were not significantly altered by chronic oral glycine treatment ($P>0.05$) (Figure 4.5).

Chronic oral glycine administration altered levels of several amino acids in liver. Levels of aspartate ($P<0.05$), glutamate ($P<0.05$) and glutamine ($P<0.05$) were decreased whereas serine ($P<0.05$) and glycine ($P<0.05$) levels were increased in the glycine-treated group. In the liver, glycine levels were increased to approximately 350% relative to the vehicle group (Figure 4.6).

Chronic oral glycine administration significantly increased glycine levels in plasma ($P<0.05$). However other plasma amino acid levels such as aspartic acid, glutamic acid, asparagine, glutamine, and serine were not significantly altered by chronic glycine treatment ($P>0.05$) (Figure 4.7).

4.4. Discussion

Chronic oral glycine administration (0.8 g/kg/day, 2 weeks) did not alter ICSS. The dose and duration of glycine used in this experiment was based on previous clinical studies suggesting improvement of schizophrenic symptoms in patients (Heresco-Levy et al. 1996). Administration of acute amphetamine (0.75 mg/kg) following 14 days of glycine treatment increased ICSS responses both in the control and glycine group. These results suggest that chronic glycine treatment may have no significant effect on brain stimulation reward in basal or amphetamine-stimulated conditions. This observation is in contrast to previous studies suggesting an antipsychotic effect of chronic glycine administration in humans (Javitt et al. 1994; Leiderman et al. 1996). However, consistent with the present result, another study found a lack of clinical efficacy of glycine and D-cycloserine in trials with healthy volunteers (D'souza et al. 2000). These authors observed that intravenous glycine administration in healthy volunteers increased glycine levels in CSF, but did not alter cognitive or acoustic startle responses. It is possible that glycine administration has no effect on normal subjects while negative symptoms of schizophrenia may be improved by the glycine therapy. VTA self-stimulation may increase mesolimbic dopamine activity which is more relevant to positive symptoms than negative symptoms of schizophrenia. Therefore, chronic oral glycine treatment may improve negative symptoms of schizophrenia but may not alter VTA self-stimulation in rats.

In accord with a previous report (Toth and Lajtha 1986), chronic glycine administration induced a small but significant increase (20%) in brain glycine levels . In

addition, glycine levels in the liver and plasma were also increased approximately 300% following this treatment. This difference may be due to a poor permeability of glycine through the blood brain barrier. Permeability of glycine into the brain is the lowest among any of the endogenous amino acids (Oldendorf 1971). Therefore, it is necessary to administer large doses of glycine to obtain significant elevations in the brain. Waziri (1996) suggested that long-term exposure to high doses of glycine may induce neurodegenerative effects by over-stimulating NMDA receptors in the brain. Other studies also reported that glycine levels are higher in the plasma and postmortem brains of schizophrenics relative to controls (Reveley et al. 1987; Waziri et al. 1993). Schizophrenia may be related to high glycine levels causing neurodevelopmental damage, as suggested by abnormal sensory gating and enlarged cerebral ventricles in hyperglycinergic (75% greater than controls) rats (Waziri and Baruah 1999).

Chronic oral glycine administration altered other amino acid levels in the liver. Serine levels were increased and aspartic acid, glutamic acid and glutamine levels were decreased in the glycine-treated group, indicating altered amino acid metabolism in the liver. Glycine and serine are converted to each other by serine hydroxymethyltransferase in the liver. Therefore, increased in glycine levels may contribute to elevated serine levels following chronic oral glycine administration. Relatively short term effects of glycine may be beneficial in improving negative symptoms, however long term effects of glycine have not been well understood. Therefore, consequences of large doses of long term glycine therapy in schizophrenia need to be carefully monitored in relation to possible toxic effects in the brain and liver.

In contrast to the NMDA receptors, the AMPA/kainate receptors may have differential effects on dopamine-mediated behaviour (Bubser et al. 1995; Svensson et al. 1995; Stephens and Cole 1996). Therefore, in the next experiment (Chapter 5), the effect of the AMPA/kainate receptor antagonist CNQX on amphetamine-stimulated spontaneous locomotor activity was investigated.

Figure 4.1. Effect of chronic glycine administration on reward threshold (M50). Glycine (0.8 g/kg/day, p.o.) or vehicle was administered and M50 was measured daily. AMPH (0.75 mg/kg, s.c.) was injected to both the glycine and vehicle group on day 14. Data shown are median (n=7). *Significant at P<0.05 relative to previous day.

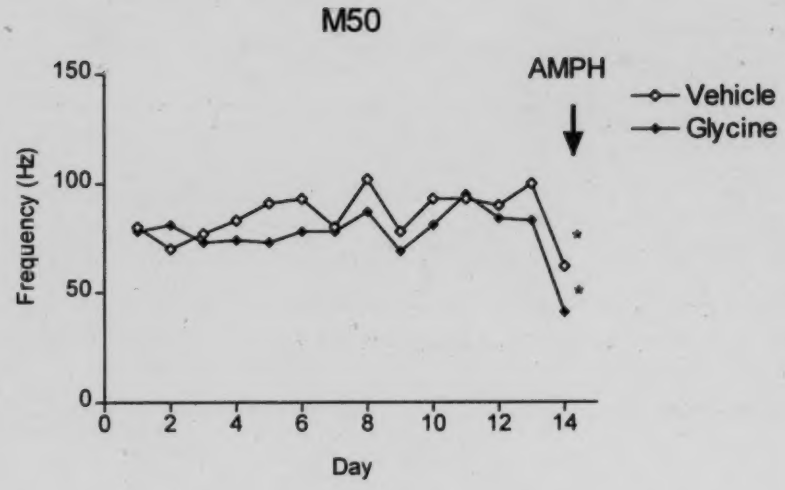


Figure 4.2. Effect of chronic glycine administration on maximum responses (RMAX). Glycine (0.8 g/kg/day, p.o.) or vehicle was administered and RMAX was measured daily. AMPH (0.75 mg/kg, s.c.) was injected to both the glycine and vehicle group on day 14. Data shown are median (n=7).

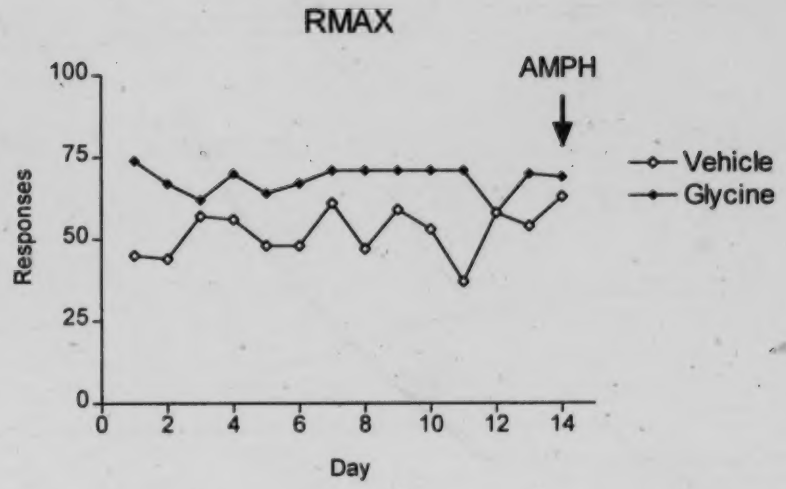


Figure 4.3. Effect of chronic glycine administration on total responses (TRES). Glycine (0.8 g/kg/day, p.o.) or vehicle was administered and TRES was measured daily. AMPH (0.75 mg/kg, s.c.) was injected to both the glycine and vehicle group on day 14. Data shown are median (n=7). *Significant at P<0.05 relative to previous day.

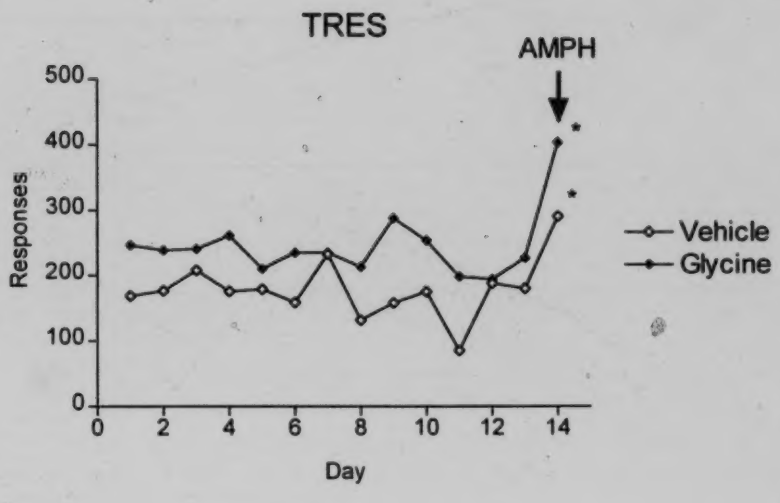


Figure 4.4. The averaged rate-frequency curves and non-linear regression curves. The vehicle and glycine curves were plotted from the data of day 13 and the vehicle+AMPH and glycine+AMPH curves were plotted from the data of day 14. Non-linear regression curves were calculated from the rate-frequency curves. Data are means±S.E.M.

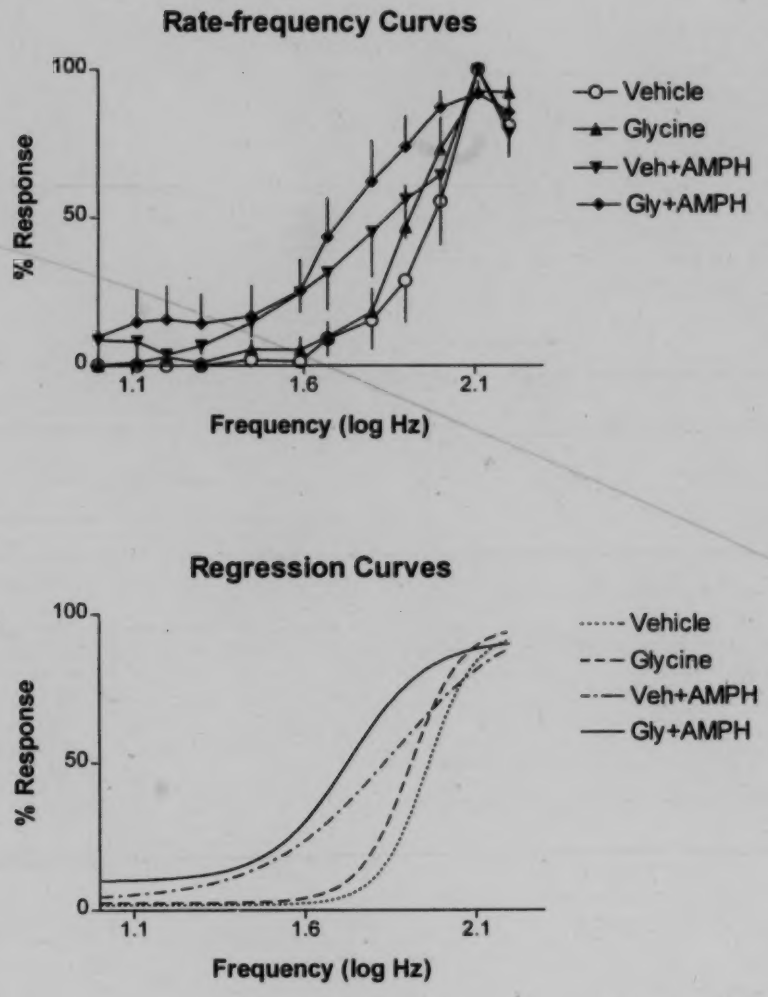


Figure 4.5. Effect of chronic oral glycine (0.8 g/kg/day, 2 weeks) on amino acid levels in the brain. Amino acid levels were analyzed using HPLC with fluorescence detection. Data are median±inter-quartile (n=7). *Significant at P<0.05, relative to the vehicle

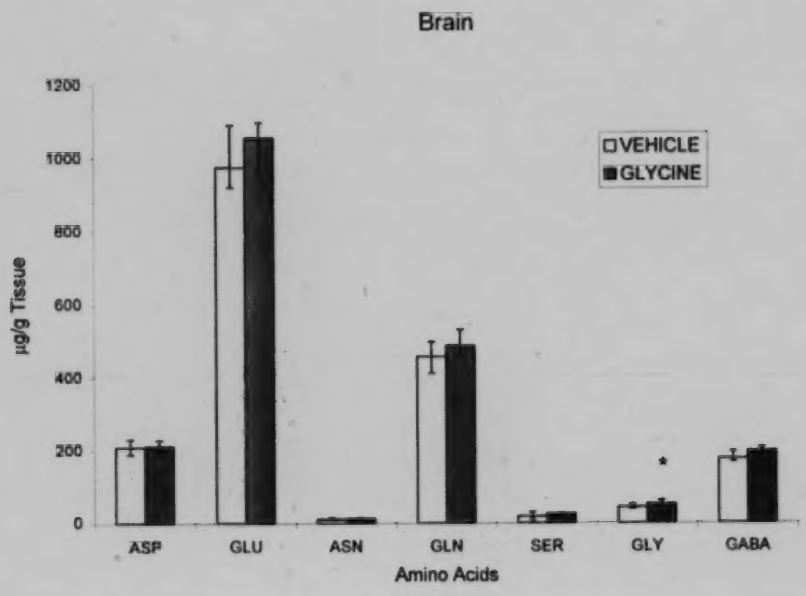


Figure 4.6. Effect of chronic oral glycine (0.8 g/kg/day, 2 weeks) on amino acid levels in the liver. Amino acid levels were analyzed using HPLC with fluorescence detection. Data are median±inter-quartile (n=7). *Significant at P<0.05, relative to the vehicle

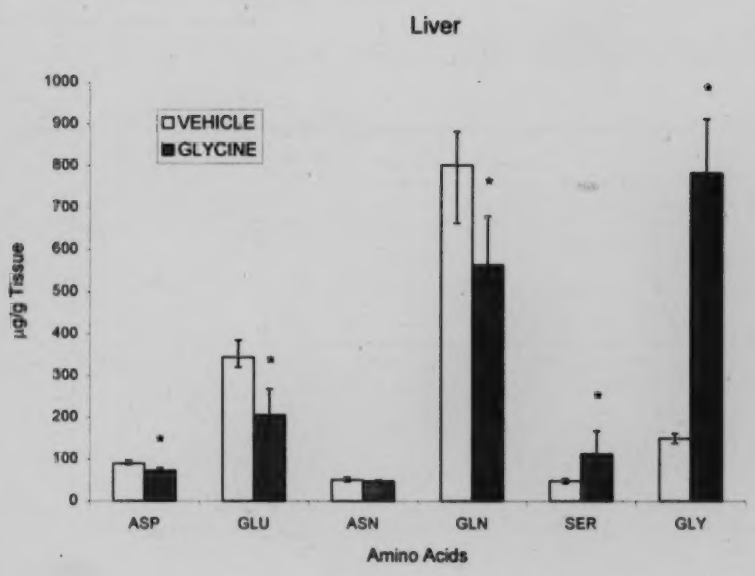
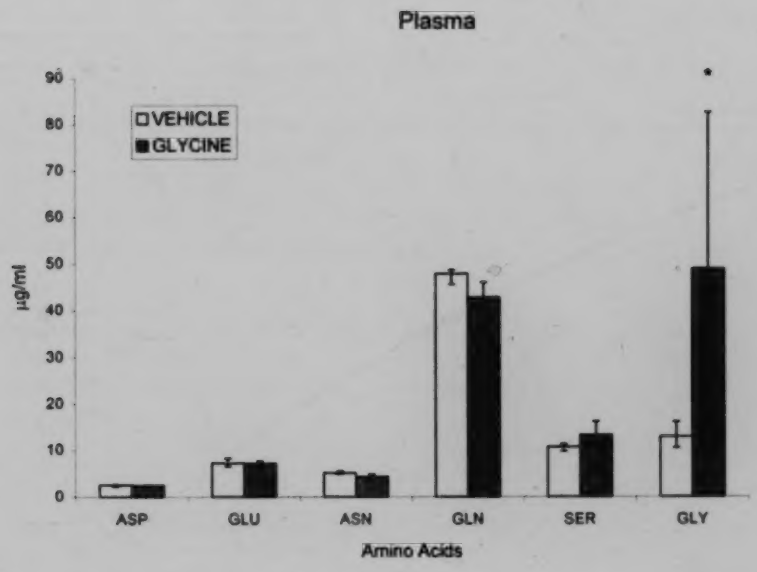


Figure 4.7. Effect of chronic oral glycine (0.8 g/kg/day, 2 weeks) on amino acid levels in plasma. Amino acid levels were analyzed using HPLC with fluorescence detection. Data are median±inter-quartile (n=7). *Significant at P<0.05, relative to the vehicle



5. EFFECTS OF CNQX ON AMPHETAMINE-INDUCED LOCOMOTOR HYPERACTIVITY

5.1. Introduction

The nucleus accumbens (NAS) in the mesolimbic system of the brain receives ascending dopaminergic and descending glutamatergic neurons originating from the ventral tegmental area (VTA) and the prefrontal cortex, respectively (Sesack and Pickel 1992; Pennartz et al. 1994). Recent studies indicate that the NAS has two major sub-regions: the core and shell. This dichotomy is based on anatomical (Voorn et al. 1986; Heimer et al. 1991), pharmacological (Deutch and Cameron 1992; Robledo and Koob 1993), electrophysiological (Rebec et al. 1997), neurochemical (Pontieri et al. 1995) and behavioural studies (Koshikawa et al. 1996; Kitamura et al. 1999). Deutch et al. (1993) proposed that the NAS shell may be related to limbic system function while the core may regulate motor function. These studies suggest structural and functional differences between the core and shell of the NAS.

AMPA receptors, which are ionotropic glutamate receptors, are multimeric proteins composed of one or more of four subunits (GluR1-4). AMPA receptors exhibit a relatively low affinity for glutamate (EC_{50}: 3-30 mM) and open quickly (<1ms) but briefly and most are selectively permeable to Na^+ (Bettler and Mulle 1995; Dingledine et al. 1999). However, certain populations of neurons may also express Ca^{2+}-permeable AMPA receptors that lack the GluR2 subunit in the brain. AMPA receptors in the NAS may be involved in the regulation of locomotion and reward-related behavior (Karler et

al. 1991; Layer et al. 1993; Kaddis et al. 1995). However, the effects of AMPA receptor-related compounds in the NAS core and shell have not been studied in detail.

CNQX is a competitive antagonist at the AMPA receptors (IC_{50} 0.3 μM) and a weak non-competitive antagonist at the glycine site of the NMDA receptors (IC_{50} 14 μM) (Sheardown et al. 1990). Administration of CNQX (approximately 0.5 μg) into the NAS stimulated horizontal and vertical locomotor activity and attenuated amphetamine-induced hyperactivity in rats (Burns et al. 1994). Svensson et al. (1995) also reported that intra-NAS administration of CNQX (0.5 μg) stimulated locomotor activity using "dopamine-depleted" mice induced by reserpine and α-methyl-para-tyrosine. Within the NAS, CNQX (0.75-1.5 μg) increased feeding behaviour when administered into the NAS shell but not when injected into the core (Maldonado-Irizarry et al. 1995). Microinjection of a high dose of CNQX (2.5 μg) into the NAS shell disrupted prepulse inhibition in rats (Wan and Swerdlow 1996).

In contrast, other studies reported a lack of effects of AMPA/kainate receptor antagonists on locomotor activity and dopamine release in the NAS. Infusion of CNQX (10 μM) into the NAS did not alter basal dopamine release in an *in vivo* microdialysis study (Imperato et al. 1990). Higher doses of CNQX (100 and 200 μM) did not alter basal dopamine release in the striatum of freely moving rats (Keefe et al. 1993; Maione et al. 1995; Finnegan and Taraska, 1996). Systemic administration of a noncompetitive AMPA antagonist, GYKI 52466 (2-4 mg/kg), did not alter locomotor activity or dopamine metabolism in the NAS (Hauber and Andersen 1993; Bubser et al. 1995). Furthermore, the selective AMPA receptor antagonist NBQX had no effect on neuroleptic-induced catalepsy (Zadow and Schmidt 1994). These studies suggest

AMPA/kainate receptor antagonists may not alter dopamine release and locomotor activity.

Amphetamine, a indirect dopamine receptor agonist, inhibits dopamine uptake and increases dopamine release in the NAS. Recent studies reported differential neurochemical and behavioural effects of amphetamine in the NAS core and shell. Greater levels of locomotor responses were observed by microinjection of amphetamine into the medial shell than when into the core of the NAS (Essman et al. 1993; Heidbreder and Feldon 1998). Amphetamine preferentially increased dopamine release in the NAS shell over the core in an *in vivo* microdialysis study (Pontieri et al. 1995). Administration of amphetamine into the NAS shell induced a conditioned place preference in rats (Schildein et al. 1998). Pierce and Kalivas (1995) reported that microinjection of amphetamine into the shell, but not into the core, of cocaine-sensitized animals is associated with a more intense behavioural response and a selective increase in amphetamine-induced extracellular dopamine levels in rats. Due to the site-selective effect of amphetamine within the NAS sub-regions, the NAS shell was examined using different doses of amphetamine (1.25-5 μg) on locomotor activity in this experiment.

Stimulation of both dopamine and glutamate receptors in the NAS may be necessary for locomotor activation mediated by psychomotor stimulants such as amphetamine and cocaine (Kaddis et al. 1993). For example, intra-NAS administration of the AMPA/kainate receptor antagonist DNQX attenuated the locomotor stimulant effect of amphetamine (Willins et al. 1992). Administration of CNQX into the NAS also attenuated locomotor hyperactivity induced by intra-NAS administration of amphetamine (Burns et al. 1994). In a prepulse inhibition study, CNQX inhibited the disruption of

prepulse inhibition induced by amphetamine in the NAS (Wan et al. 1995). In a conditioned place preference study, CNQX prevented place preference induced by amphetamine in mice (Mead and Stephens 1999). In an *in vivo* microdialysis study, CNQX attenuated elevated extracellular dopamine levels induced by cocaine in the rat striatum (Moghaddam and Bolinao 1994). Another AMPA receptor antagonists, GYKI 52466 and NBQX, attenuated locomotor hyperactivity and stereotypy induced by amphetamine in mice (Vanover 1998). Therefore, AMPA/kainate receptor antagonists may induce inhibitory effects on psychostimulant-induced dopamine release and behaviour.

The purpose of this experiment was to investigate the effects of CNQX on amphetamine-stimulated locomotor activity following administration into the NAS core and shell. In the first experiment, different doses of CNQX (0.25 and 0.5 μg) were administered into the NAS core and shell and locomotor activity was monitored. In the second experiment, different doses of amphetamine (1.25, 2.5 and 5 μg) were administered into the NAS shell and locomotor activity was monitored. In the third experiment, CNQX (0.5 μg) and amphetamine (5 μg) were co-administered into the NAS shell and locomotor activity was monitored. It was expected that administration of amphetamine into the NAS shell may increase locomotor activity in a dose-dependent manner. It was hypothesized that CNQX may inhibit amphetamine-induced hyperactivity when these compounds were co-administered into the NAS shell.

5.2. Methods

5.2.1. Microinjection of CNQX into the NAS Core and Shell

Male Sprague-Dawley rats (250-300 g) were implanted with bilateral guide cannulae in the NAS core (n=6) or the shell (n=6). One week after surgery, animals were handled and habituated to the photobeam activity boxes for 1 hr daily for three days. Following the habituation period, each animal received three microinjections comprised of vehicle, CNQX 0.25 μg or 0.5 μg with a three day interval between each microinjection. Following each microinjection, each animal was placed in a photobeam activity box and spontaneous locomotor activity was monitored for 60 min. The CNQX:HBC complex was dissolved in 0.45% saline to obtain 4 mM (1 μg/μl) CNQX. The HBC (45%) was dissolved in 0.45% saline and used as a vehicle.

5.2.2. Microinjection of Amphetamine into the NAS Shell

Male Sprague-Dawley rats (250-300 g) were implanted with bilateral guide cannulae in the NAS shell (n=10). One week after surgery, animals were handled and habituated to the photobeam activity boxes for 1 hr daily for three days. Following the habituation period, each animal received four microinjections comprising vehicle (aCSF, pH 7.2), amphetamine 1.25 μg, 2.5 μg or 5 μg with a three day interval between each microinjection. Following each microinjection, each animal was placed in a photobeam activity box and spontaneous locomotor activity was monitored for 60 min. (+)-Amphetamine sulfate was dissolved in 0.9% saline.

5.2.3. Microinjection of CNQX and Amphetamine into the NAS shell

Male Sprague-Dawley rats (250-300 g) were implanted with bilateral cannulae in the NAS shell (n=8). One week after surgery, animals were handled and habituated to the photobeam activity boxes for 1 h daily for three days. Following the habituation period, each animal received four microinjections comprised of vehicle, CNQX 0.5 μg, amphetamine 5 μg and a mixture of CNQX and amphetamine with a three day interval between each microinjection. Following each microinjection, each animal was placed in a photobeam activity box and spontaneous locomotor activity was monitored for 60 min. The CNQX:HBC complex was dissolved in 0.45% saline and (+)-amphetamine sulfate was dissolved in 45% HBC solution. The 45% HBC aqueous solution was used as a vehicle.

5.2.4. Statistics

Either CNQX or amphetamine dose-response data were analyzed using two-way ANOVA (drug × time) with repeated-measures. A combination of CNQX and amphetamine data was analyzed using three way ANOVA (CNQX × amphetamine × time) with repeated-measures. Local time course data were analyzed using one-way ANOVA across all treatments at each 5-min interval. A significant F-ratio ($P<0.05$) on any 5-min interval was followed by a comparison of each drug treatment with vehicle using Tukey's test.

5.3. Results

5.3.1. Effects of CNQX on Locomotor Activity

Administration of CNQX (0.25, 0.5 μg) into the NAS shell did not alter total activity [$F(2,10)=0.133$, $P>0.05$] and there was no significant interaction between CNQX and time [$F(22,110)=0.524$, $P>0.05$]. The local time-course data (line graph) show no significant differences between the control and doses of CNQX at each 5 min of the testing period (Figure 5.1).

Administration of CNQX (0.25, 0.5 μg) into the NAS core did not alter total activity [$F(2,10)=0.536$, $P>0.05$] and there was no significant interaction between CNQX and time [$F(22,110)=0.624$, $P>0.05$]. The local time course data (line graph) show no significant differences between the control and doses of CNQX at each 5 min of the testing period (Figure 5.2).

5.3.2. Effects of Amphetamine on Locomotor Activity

Administration of amphetamine (1.25, 2.5 or 5 μg) into the NAS shell significantly increased total activity [$F(3,27)=13.333$, $P<0.05$]. *Post-hoc* tests ($P<0.05$) performed on the individual mean values revealed significant hyperactivity at each dose compared to vehicle treatment. There was a significant interaction of amphetamine × time [$F(33,297)=2.962$, $P<0.05$]. The local time-course data (line graph) showed significant hyperactivity of amphetamine (5 μg) during 15 and 45 min of testing compared to the control (Figure 5.3).

Administration of amphetamine (1.25, 2.5 or 5 μg) into the NAS shell increased vertical activity [F (3,27)=3.273, P<0.05]. *Post-hoc* tests (P<0.05) performed on the individual mean values revealed significant hyperactivity at the 1.25 and 5 μg doses compared to the control. A significant interaction of amphetamine × time [F (33,297)=1.726, P<0.05] was found. The local time-course data (line graph) showed overall hyperactivity of amphetamine (5 μg) but multiple comparisons tests at each 5 min did not show significant differences compared to the control, except at 35 min of testing period (Figure 5.4).

Administration of amphetamine (1.25, 2.5 or 5 μg) into the NAS shell did not affect consecutive activity [F (3,27)=1.562, P>0.05]. However there was a significant interaction of amphetamine × time [F (33,297)=2.032, P<0.05]. The local time course data (line graph) showed amphetamine (2.5 and 5 μg) significantly increased consecutive activity during 35-45 min of the testing period relative to the control (Figure 5.5).

5.3.3. Effects of CNQX on Amphetamine-induced Hyperactivity

Administration of CNQX (0.5 μg) into the NAS shell did not alter total activity [F(1,7)=0.002, P>0.05]. Administration of amphetamine (5 μg) into the NAS shell increased total activity [F(1,7)=14.589, P<0.05]. Following co-administration of CNQX and amphetamine there was no significant interaction on total activity [F(1,7)=2.745, P>0.05]. However there was a significant interaction of CNQX × amphetamine × time on total activity [F(11,77)=2.442, P<0.05]. The local time-course data (line graph) revealed that administration of a mixture of CNQX and amphetamine tended to inhibit amphetamine-hyperactivity during the first 20 min of testing. However, multiple

comparisons tests at each 5 min of the testing period did not show significant differences between amphetamine treatment and a mixture of CNQX and amphetamine treatment (Figure 5.6).

Administration of CNQX (0.5 μg) into the NAS shell did not alter vertical activity [$F(1,7)=0.955$, $P>0.05$]. Amphetamine (5 μg) significantly increased vertical activity [$F(1,7)=10.094$, $P<0.05$]. Co-administration of CNQX and amphetamine into the NAS shell had no significant interaction [$F(1,7)=0.219$, $P>0.05$] and there was no interaction of CNQX × amphetamine × time [$F(11,77)=0.848$, $P>0.05$]. The local time-course data (line graph) showed significant hyperactivity during the later period of testing (45 and 60 min) with a mixture of CNQX and amphetamine treatment (Figure 5.7).

Administration of CNQX (0.5 μg) into the NAS shell did not alter consecutive activity [$F(1,7)=3.668$, $P>0.05$]. Amphetamine (5 μg) significantly increased consecutive activity [$F(1,7)=6.744$, $P<0.05$]. Co-administration of CNQX and amphetamine into the NAS shell had no interaction [$F(1,7)=0.780$, $P>0.05$]. No significant interaction of CNQX × amphetamine × time was found [$F(11,77)=0.552$, $P>0.05$]. The local time-course data (line graph) illustrate the lack of significant differences between each treatment at each 5 min testing period. However a combination of CNQX and amphetamine induced a late hyperactivity at the 50 and 60 min testing periods (Figure 5.8).

5.4. Discussion

Administration of CNQX (0.25 or 0.5 μg) into the NAS core and shell did not alter spontaneous locomotor activity. This result is consistent with previous studies suggesting that AMPA/kainate receptor antagonists are behaviourally inactive in "dopamine intact" animals (Hauber and Andersen 1993; Danysz et al. 1994). However, higher doses of CNQX (>0.5 μg) were not tested in this experiment due to the low solubility of the CNQX:HBC complex in aqueous solution; maximum solubility of this compound was approximately 0.5 μg/0.5 μl. Therefore, it is possible that higher doses of CNQX (>0.5 μg) may alter behaviour as reported in the previous study (Wan and Swerdlow 1996). These authors reported that a high dose of CNQX (2.5 μg) into the NAS shell disrupted the prepulse inhibition in rats. CNQX was dissolved in 0.1 N sodium hydroxide and adjusted by 0.1 N hydrogen chloride to a pH range for 5-7 in that study. However, these authors suggested that this effect of CNQX (2.5 μg) may have been mediated by blockade of the glycine binding site of the NMDA receptors.

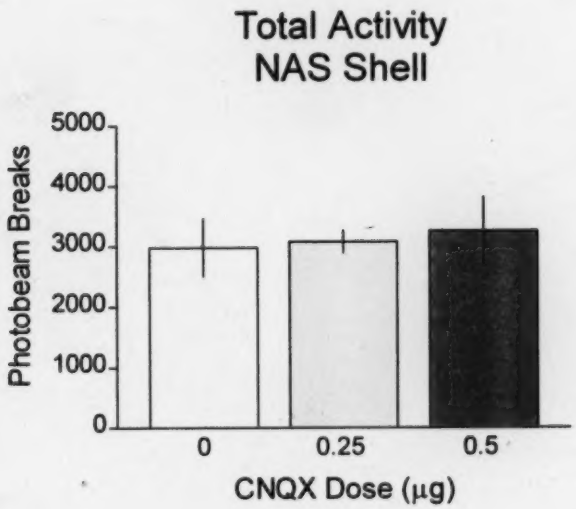
Administration of amphetamine (1.25, 2.5 or 5 μg) into the NAS shell significantly increased spontaneous locomotor activity in a dose-dependent manner. It has been suggested that the NAS may be a major target area involved in locomotor stimulant effects of amphetamine (Vezina and Stewart 1990; Bardo 1998). Several studies demonstrated that locomotor responses to amphetamine are greater in the shell than the core of the NAS (Essman et al. 1993; Ranaldi and Beninger 1994; Heidbreder and Feldon 1998). In agreement with these studies, present doses of amphetamine (1.25-5 μg) significantly increased locomotor activity when administered into the NAS shell in

the present experiment. The greater response of amphetamine in the NAS shell suggests that activation of both D1-like and D2-like dopamine receptors in the NAS shell may be necessary for locomotor stimulation.

Co-administration of CNQX and amphetamine into the NAS shell did not alter overall amphetamine hyperactivity (60 min). However, this combination attenuated the early phase (20 min) of amphetamine hyperactivity. This inhibitory effect of CNQX was statistically significant as revealed by interaction of CNQX × amphetamine × time on total locomotor activity. Kelley and Throne (1992) also reported inhibitory effects of the NMDA receptor antagonist AP-5 (0.5 μg) on amphetamine (5 μg) hyperactivity following co-administration into the NAS. This effect was only significant in first 30 min of the testing period as shown in the similar pattern of local time course analysis in that study. Consistent with the present result, other studies reported the inhibitory effects of AMPA/kainate receptor antagonists on hyperactivity induced by psychostimulants (Kaddis et al. 1993; Burns et al. 1994; Svensson et al. 1995).

Repeated administration of psychostimulants induce enhanced responses on locomotor activity. Recent studies suggested that acute or repeated administration of amphetamine or cocaine may induce differential effects on glutamate transmission in the NAS (Pierce and Kalivas 1997; Wolf 1998). Therefore, the effect of CNQX on locomotor activity was investigated following repeated administration of amphetamine into the NAS shell in the next experiment (Chapter 6).

Figure 5.1. Effect of intra-NAS shell administration of CNQX on total activity. CNQX (0, 0.25 or 0.5 μg/side, bilateral) was microinjected into the NAS shell and total locomotor activity was monitored for 60 minutes. Data are means±S.E.M. (n=6)



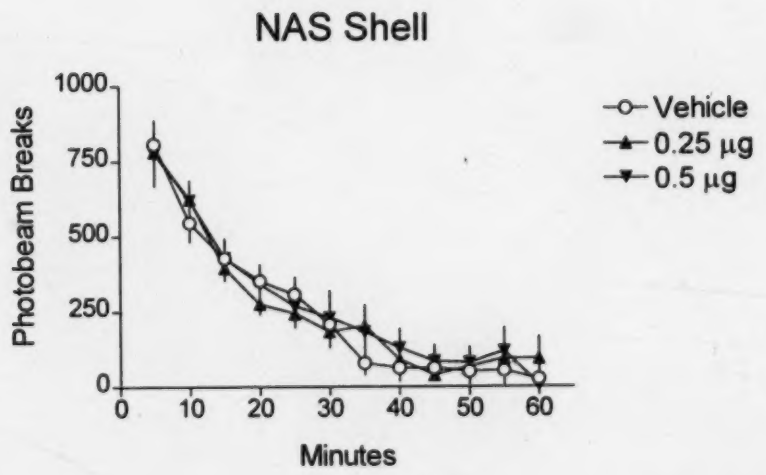
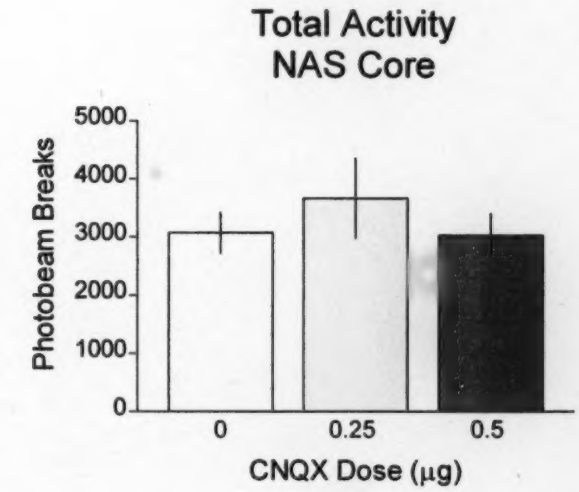


Figure 5.2. Effect of intra-NAS core administration of CNQX on total activity. CNQX (0, 0.25 or 0.5 μg/side, bilateral) was microinjected into the NAS core and total locomotor activity was monitored for 60 minutes. Data are means±S.E.M. (n=6)



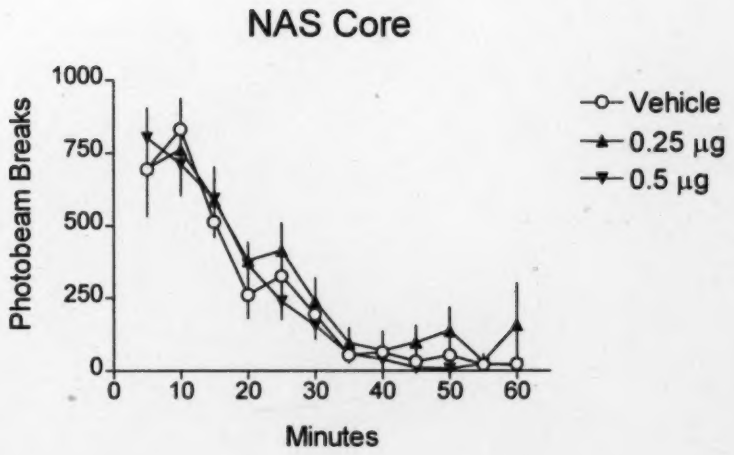
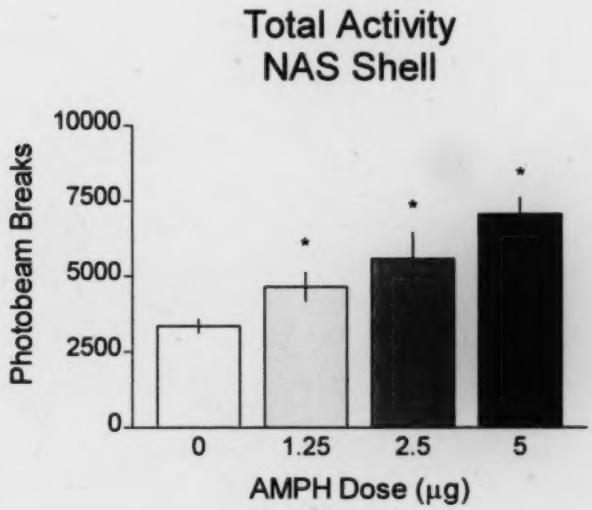


Figure 5.3. Effect of intra-NAS shell administration of AMPH on total activity. AMPH (0, 1.25, 2.5 or 5 μg/side, bilateral) was microinjected into the NAS shell and total activity was monitored for 60 minutes. Data are means±S.E.M. (n=10) *Significant at P<0.05, relative to vehicle.



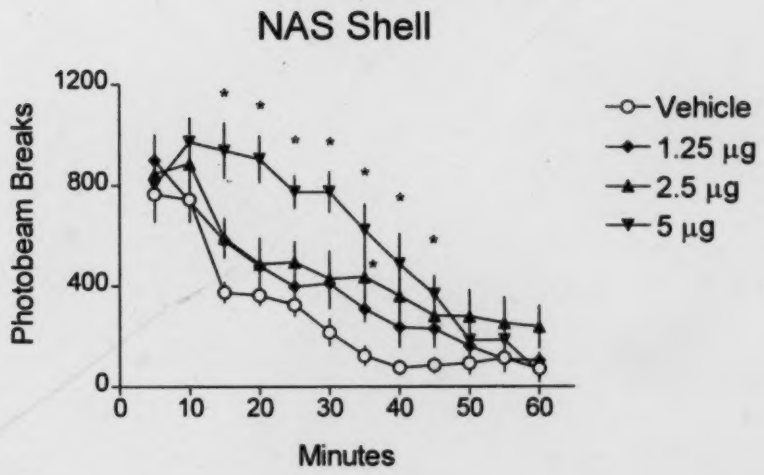
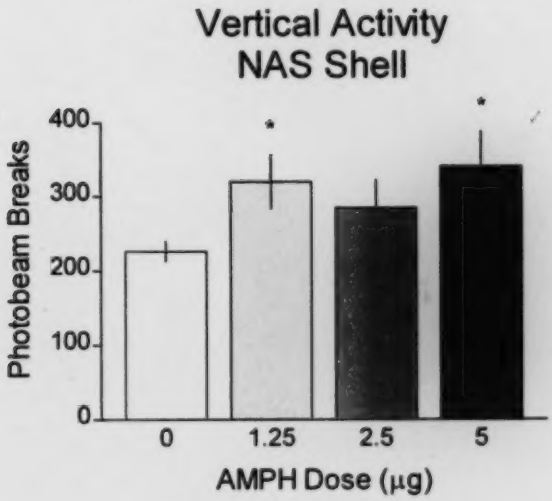


Figure 5.4. Effect of intra-NAS shell administration of AMPH on vertical activity. AMPH (0, 1.25, 2.5 or 5 μg/side, bilateral) was microinjected into the NAS shell and vertical activity was monitored for 60 minutes. Data are means±S.E.M. (n=10) *Significant at P<0.05, relative to vehicle.



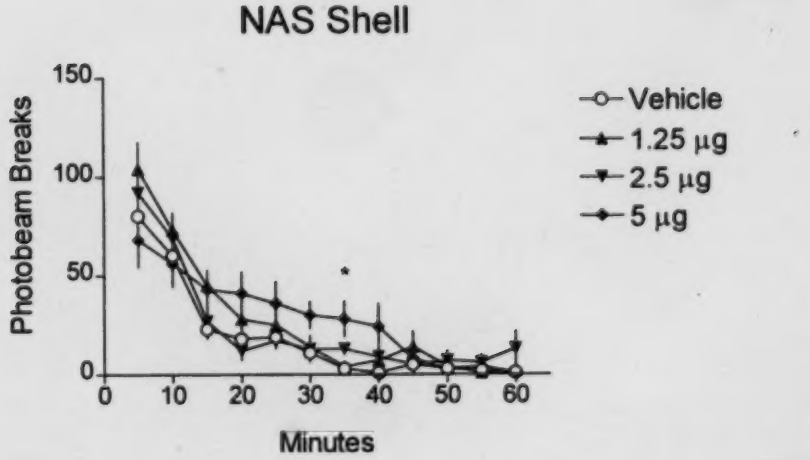
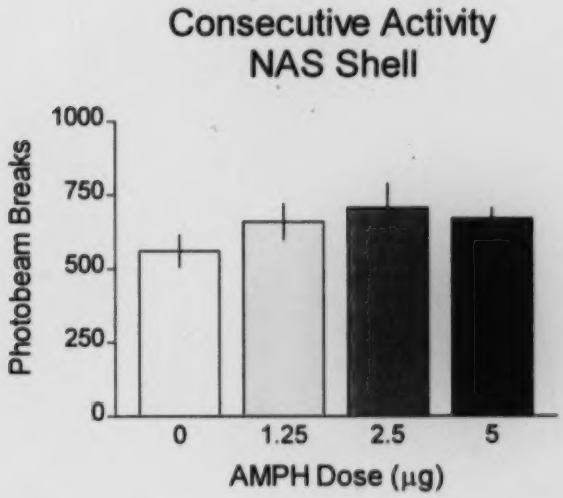


Figure 5.5. Effect of intra-NAS shell administration of AMPH on consecutive activity. AMPH (0, 1.25, 2.5 or 5 μg/side, bilateral) was microinjected into the NAS shell and consecutive activity was monitored for 60 minutes. Data are means±S.E.M. (n=10) *Significant at P<0.05, relative to vehicle.



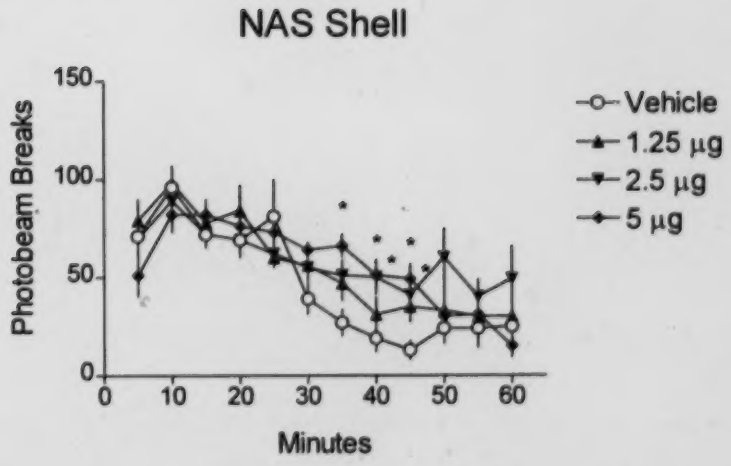
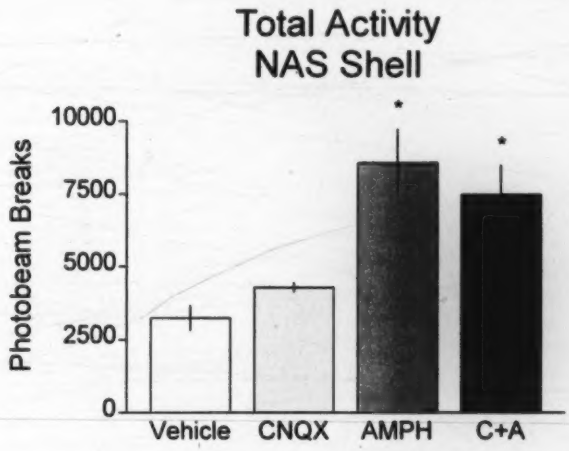


Figure 5.6. Effect of CNQX in the NAS shell on AMPH-induced total activity. CNQX (0.5 μg) and AMPH (5 μg) were microinjected into the NAS shell and total activity was monitored for 60 minutes. Data are means±S.E.M. (n=8) *Significant at P<0.05, relative to vehicle.



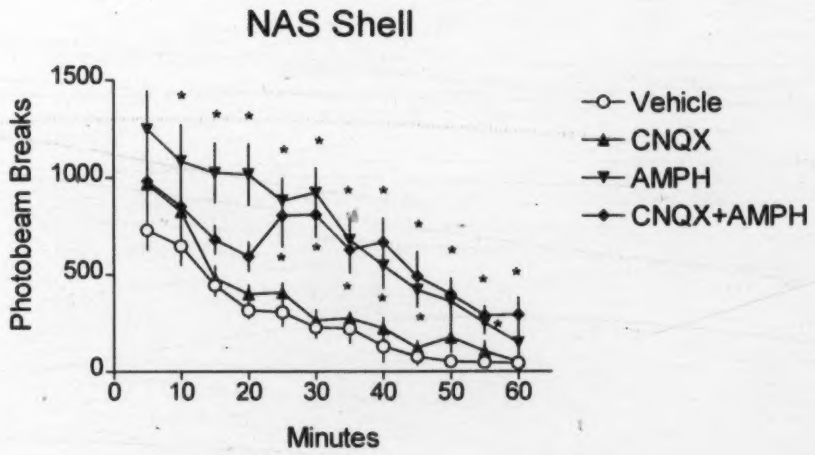
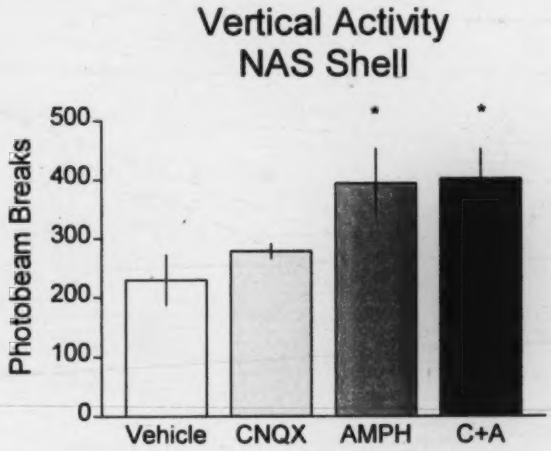


Figure 5.7. Effect of CNQX in the NAS shell on AMPH-induced vertical activity. CNQX (0.5 μg) and AMPH (5 μg) were microinjected into the NAS shell and vertical activity was monitored for 60 minutes. Data shown are means±S.E.M. (n=8) *Significant at $P<0.05$, relative to vehicle.



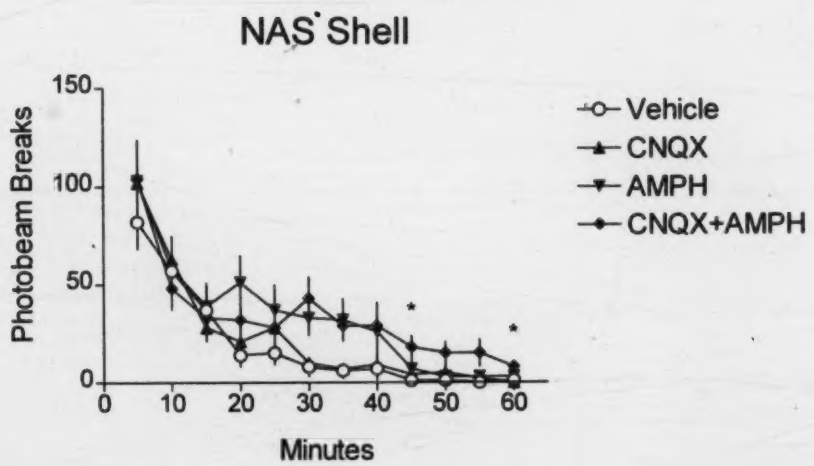
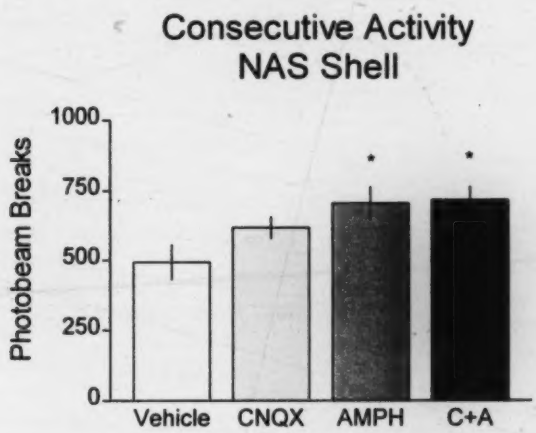
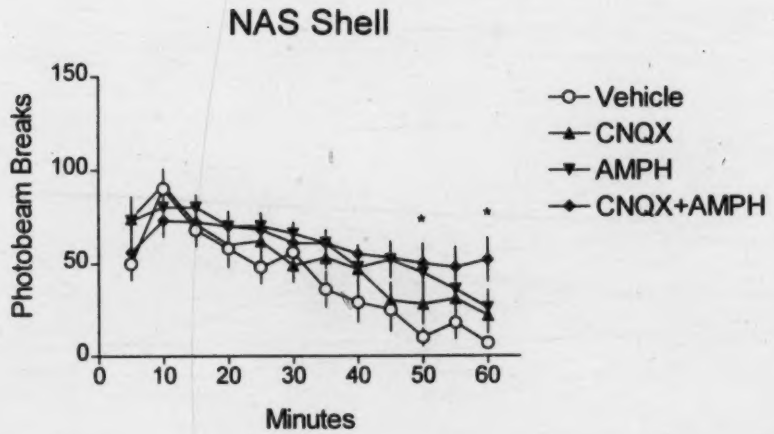


Figure 5.8. Effect of CNQX in the NAS shell on AMPH-induced consecutive activity. CNQX (0.5 μg) and AMPH (5 μg) were microinjected into the NAS shell and consecutive activity was monitored for 60 minutes. Data shown are means±S.E.M. (n=8) *Significant at P<0.05, relative to vehicle.





6. EFFECTS OF CNQX ON REPEATED AMPHETAMINE-INDUCED LOCOMOTOR HYPERACTIVITY

6.1. Introduction

Acute or repeated systemic administration of psychostimulants, such as amphetamine and cocaine, may induce differential effects on glutamate function in the NAS (see Pierce and Kalivas 1997; Wolf 1998). In the previous experiment (Chapter 5), co-administration of CNQX into the NAS shell attenuated hyperactivity induced by amphetamine. However, this inhibitory effect of CNQX was only evident in the first 20 min of testing as revealed in the local time course analysis. It has been reported that acute or repeated administration of amphetamine may elevate extracellular glutamate levels in the NAS by different mechanisms (Xue et al. 1996). Other studies also reported that repeated systemic administration of amphetamine or cocaine may alter AMPA receptor expression in the NAS (Lu et al. 1997; Churchill et al. 1999; Kelz et al. 1999). The NAS has been suggested as a major target area of amphetamine action following systemic administration of amphetamine (Bardo 1998). Therefore, it is possible that repeated administration of amphetamine into the NAS may induce differential effects on glutamate function relative to acute administration of amphetamine.

Repeated administration of psychostimulants has been reported to develop enhanced behavioural responses, also termed behavioural sensitization (Robinson and Becker 1986). Dopamine and glutamate receptors in the mesolimbic system may play a role in psychostimulant-induced behavioral sensitization (see Wolf 1998). The NAS has

been implicated in expression of behavioural sensitization induced by amphetamine (Vezina and Stewart 1990; Cador et al. 1995). Karler et al. (1991) reported that the AMPA/kainate receptor antagonist DNQX inhibited stereotypy related to amphetamine behavioural sensitization. DNQX may also prevent the development of behavioural sensitization induced by amphetamine or cocaine, indicating an involvement of AMPA/kainate receptors in long-term psychostimulant effects (Karler et al. 1994; 1995). Administration of NBQX also prevented the development of amphetamine sensitization-related locomotor hyperactivity, but not the expression of sensitization (Li et al. 1997). Administration of CNQX into the NAS core prevented expression of cocaine sensitization after 14 and 23 days of withdrawal (Pierce et al. 1996). Taken together, these studies suggest that AMPA/kainate receptor antagonists may prevent behavioural sensitization mediated by psychostimulants such as cocaine and amphetamine.

The purpose of this experiment was to investigate the effects of CNQX on hyperactivity induced by repeated administration of amphetamine into the NAS shell. It has been reported that repeated administration of amphetamine into the VTA, but not into the NAS, resulted in the development of behavioural sensitization (Vezina and Stewart 1990; Cardo et al. 1995). Although the NAS has been implicated in the expression, but not in the development, of amphetamine sensitization, a substantial amount of evidence suggests a significant role of the NAS in psychostimulant-induced behavioural sensitization (Pierce and Kalivas 1997; Wolf 1998). CNQX induced small and transient inhibitory effects on acute amphetamine-induced hyperactivity (Chapter 5). Therefore, the effects of CNQX on repeated amphetamine-induced hyperactivity were further investigated in this experiment.

6.2. Methods

6.2.1. Microinjection of Repeated Amphetamine followed by Acute CNQX in the NAS Shell

Male Sprague-Dawley rats (250-300 g) were implanted with bilateral guide cannulae in the NAS shell (n=32). One week after surgery, the animals were randomized into four groups consisting of vehicle, CNQX, amphetamine and a combination of CNQX and amphetamine (n=8 per group). The animals were handled and habituated to the photobeam activity boxes for 1 hour daily for three days. Following the habituation period, each group of animals received three repeated microinjections of each treatment (vehicle, CNQX 0.5 μg, amphetamine 5 μg and a mixture of CNQX and amphetamine) with a three day interval between each microinjection. On the fourth day of microinjection, all groups of animals received a combination of CNQX and amphetamine treatment into the NAS shell. Following each set of microinjections, each animal was placed in a photobeam activity box and spontaneous locomotor activity was monitored for 60 min. The CNQX:HBC complex was dissolved in 0.45% saline and (+)-amphetamine sulfate was dissolved in 45% HBC solution. The 45% HBC aqueous solution was used as a vehicle.

6.2.2. Statistics

Locomotor activity data were analyzed using three-way ANOVA with mixed design (group × day × time) followed by *post-hoc* multiple comparisons of means using Tukey's test. Local time course data were analyzed using two-way ANOVA with

repeated measures (day × time). A significant F-ratio ($P<0.05$) on any 5-min interval was followed by multiple comparisons of means using Tukey's test.

6.3. Results

6.3.1. Effects of CNQX on Repeated Amphetamine-induced Locomotor Hyperactivity

The effects of CNQX on repeated amphetamine-induced hyperactivity are represented as total activity (Figure 6.1), vertical activity (Figure 6.2) and consecutive activity (Figure 6.3), respectively.

Administration of amphetamine (5 μg) or a combination of CNQX (0.5 μg) and amphetamine (5 μg) into the NAS shell significantly increased total activity compared to the vehicle group on microinjection day 1 [$F(1,3)=9.027$, $P<0.05$], day 2 [$F(1,3)=11.601$, $P<0.05$] and day 3 [$F(1,3)=10.995$, $P<0.05$]. There was no significant difference between microinjection days [$F(6,56)=1.643$, $P>0.05$] because each group received the same treatment during microinjection day 1-day 3. A comparison between microinjection day 3 and day 4 revealed significant interaction (day and group) [$F(3,28)=12.142$, $P<0.05$]. *Post-hoc* tests showed that total activity was significantly increased in vehicle and CNQX groups on microinjection day 4 (Figure 6.1).

Administration of amphetamine (5 μg) or a combination of CNQX (0.5 μg) and amphetamine (5 μg) into the NAS shell significantly increased vertical activity compared to the vehicle group on microinjection day 1 [$F(1,3)=5.431$, $P<0.05$], day 2 [$F(1,3)=6.680$, $P<0.05$] and day 3 [$F(1,3)=4.508$, $P<0.05$]. There was no significant

difference between microinjection days (day 1-3) [$F(6,56)=0.406$, $P>0.05$]. A comparison between microinjection day 3 and day 4 revealed significant interaction (day and group) [$F(3,28)=9.295$, $P<0.05$]. *Post-hoc* tests showed that vertical activity was significantly increased in the vehicle group on microinjection day 4 (Figure 6.2).

Administration of amphetamine (5 μg) or a combination of CNQX (0.5 μg) and amphetamine (5 μg) into the NAS shell significantly increased consecutive activity compared to the vehicle group on microinjection day 1 [$F(1,3)=9.244$, $P<0.05$], day 2 [$F(1,3)=13.168$, $P<0.05$] and day 3 [$F(1,3)=12.183$, $P<0.05$]. There was no significant difference between microinjection days (day 1-3) [$F(6,56)=1.057$, $P>0.05$]. A comparison between microinjection day 3 and day 4 revealed significant interaction (day and group) [$F(3,28)=8.565$, $P<0.05$]. *Post-hoc* tests showed that consecutive activity was significantly increased in vehicle and CNQX groups on microinjection day 4 (Figure 6.3).

The local time course data represent the effects of a combination of CNQX and amphetamine on locomotor activity in the vehicle group (Figure 6.4), CNQX group (Figure 6.5), amphetamine group (Figure 6.6) and the combined CNQX and amphetamine treatment group (Figure 6.7).

The vehicle group showed significant hyperactivity following administration of the mixture of CNQX and amphetamine treatment on microinjection day 4: total activity [$F(33,231)=1.503$, $P<0.05$] and consecutive activity [$F(33,231)=1.506$, $P<0.05$] but not vertical activity [$F(33,231)=1.152$, $P>0.05$]. The local time course analysis at each 5 min revealed this combination significantly increased total and vertical activity 30 min after microinjection (Figure 6.4).

The CNQX group exhibited significant hyperactivity following combined administration of CNQX and amphetamine on microinjection day 4: total activity [$F(33,231)=3.118$, $P<0.05$], vertical activity [$F(33,231)=2.360$, $P<0.05$] and consecutive activity [$F(33,231)=2.491$, $P<0.05$]. The local time course analysis at each 5 min interval revealed that this combination significantly increased total, vertical and consecutive activity (Figure 6.5).

The amphetamine group revealed a significant interaction between microinjection day and time following administration of the mixture of CNQX and amphetamine on microinjection day 4: total activity [$F(33,231)=2.904$, $P<0.05$], vertical activity [$F(33,231)=1.870$, $P<0.05$], but not consecutive activity [$F(33,231)=0.973$, $P>0.05$]. However, the local time course analysis at each 5 min did not show significant differences between microinjection days (Figure 6.6).

The group receiving a combination of CNQX and amphetamine on each day showed no significant differences between microinjections days. This group received the same treatment for all microinjection days (day 1-4): total activity [$F(33,231)=0.886$, $P>0.05$], vertical activity [$F(33,231)=0.783$, $P>0.05$] and consecutive activity [$F(33,231)=0.783$, $P>0.05$]. The local time course data at each 5 min showed no difference between microinjection days (Figure 6.7).

6.4. Discussion

Repeated administration of CNQX (0.5 μg) into the NAS shell did not alter locomotor activity. This result is consistent with the previous experiment (Chapter 5)

indicating a lack of motor effect of CNQX when administered into the NAS core and shell. These results provide further evidences that blockade of AMPA/kainate receptors in the NAS of "dopamine-intact" animals may have no significant effect on locomotor activity. Repeated administration of amphetamine (5 μg) into the NAS shell increased locomotor activity, but did not cause development of behavioural sensitization. Previous studies also reported a lack of a behavioural sensitization effect following repeated administration of amphetamine into the NAS (Vezina and Stewart 1990; Cador et al. 1995). These authors demonstrated that repeated administration of amphetamine into the VTA induced development of sensitization, whereas repeated injections into the NAS were sufficient to maintain the sensitized response.

Co-administration of CNQX did not alter overall hyperactivity (60 min) induced by repeated administration of amphetamine (Figure 6.1). However, CNQX tended to inhibit the early phase (first 20 min) of amphetamine-induced hyperactivity (Figure 6.6) and there was significant interaction between microinjection day and time. These small inhibitory effects of CNQX are similar to those observed in the previous experiment (chapter 5). Inhibitory effects of AMPA/kainate receptor antagonists on psychostimulant-induced behavioural sensitization was based on systemic administration of these compounds (Karler et al. 1991; 1994). The present study suggests that the blockade of AMPA/kainate receptors in the NAS shell may attenuate the hyperactivity induced by acute or repeated administration of amphetamine into the NAS shell.

Although the AMPA/kainate receptor antagonist CNQX induced a small inhibitory effect on intra-NAS shell amphetamine-induced hyperactivity, it is difficult to predict which subtypes of dopamine receptors are mainly involved in the interaction with

CNQX due to indirect action of amphetamine on different dopamine receptors. A recent study suggested that glutamate-dopamine interaction in the NAS may depend on relative involvement of D1-like and D2-like dopamine receptors in the NAS (Svensson et al. 1994a). Therefore, in the next experiment (Chapter 7), CNQX was tested with 7-OH-DPAT to investigate the possible interaction between the AMPA/kainate receptors and the D2/D3 dopamine receptors in the NAS core and shell.

Figure 6.1. Effect of CNQX (0.5 μg) on the effects of repeated AMPH (5 μg) in the NAS shell on total activity. Each group received three repeated microinjections (day 1-3) then all groups received a mixture of CNQX and AMPH on microinjection day 4. Following each microinjection, total activity was monitored for 60 min. Data are means±S.E.M. (n=8 per group) *Significant at P<0.05, relative to vehicle group. #Significant at P<0.05, relative to microinjection day 3.

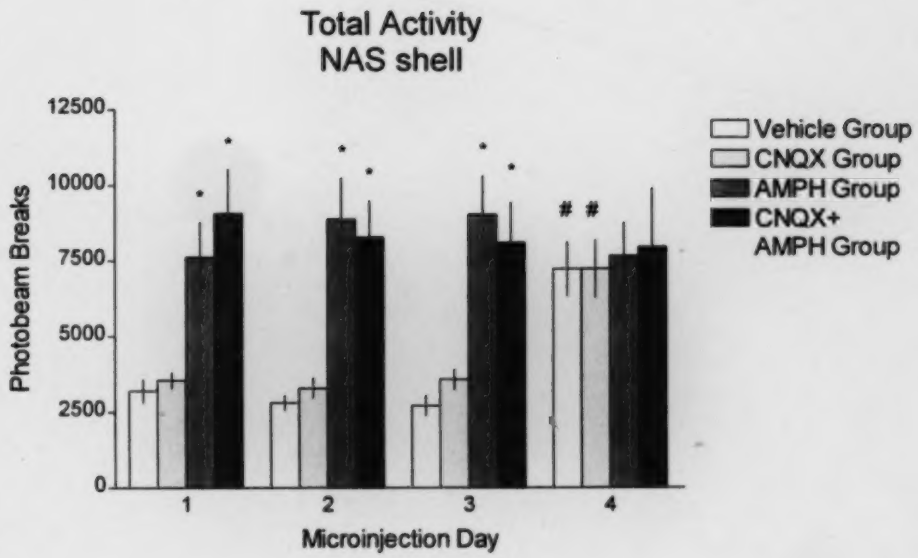


Figure 6.2. Effect of CNQX (0.5 μg) on the effect of repeated AMPH (5 μg) in the NAS shell on vertical activity. Each group received three repeated microinjections (day 1-3) then all groups received a mixture of CNQX and AMPH on microinjection day 4. Following each microinjection, vertical activity was monitored for 60 min. Data are means±S.E.M. (n=8 per group) *Significant at P<0.05, relative to vehicle group. #Significant at P<0.05, relative to day 3.

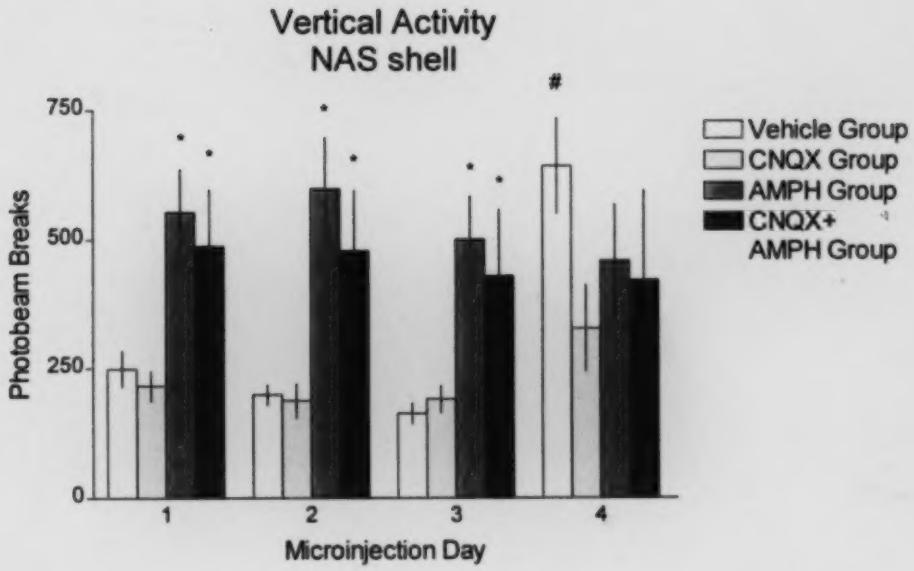


Figure 6.3. Effect of CNQX (0.5 μg) on the effect of repeated AMPH (5 μg) in the NAS shell on consecutive activity. Each group received three repeated microinjections (day 1-3) then all groups received a mixture of CNQX and AMPH on microinjection day 4. Following each microinjection, consecutive activity was monitored for 60 min. Data are means±S.E.M. (n=8 per group) *Significant at P<0.05, relative to vehicle group. #Significant at P<0.05, relative to day 3.

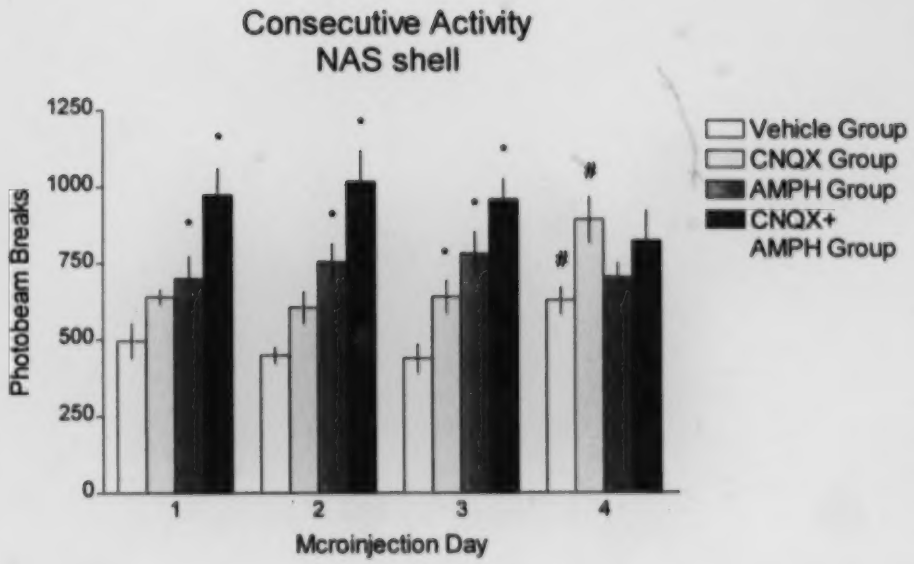


Figure 6.4. Effect of a mixture of CNQX and AMPH on repeated vehicle-treated group. Animals received three repeated vehicle microinjections into the NAS shell (day 1-3) followed by a mixture of CNQX (0.5 μg) and AMPH (5 μg) injection on day 4. Data are means±S.E.M. (n=8) *Significant at P<0.05, relative to day 3.

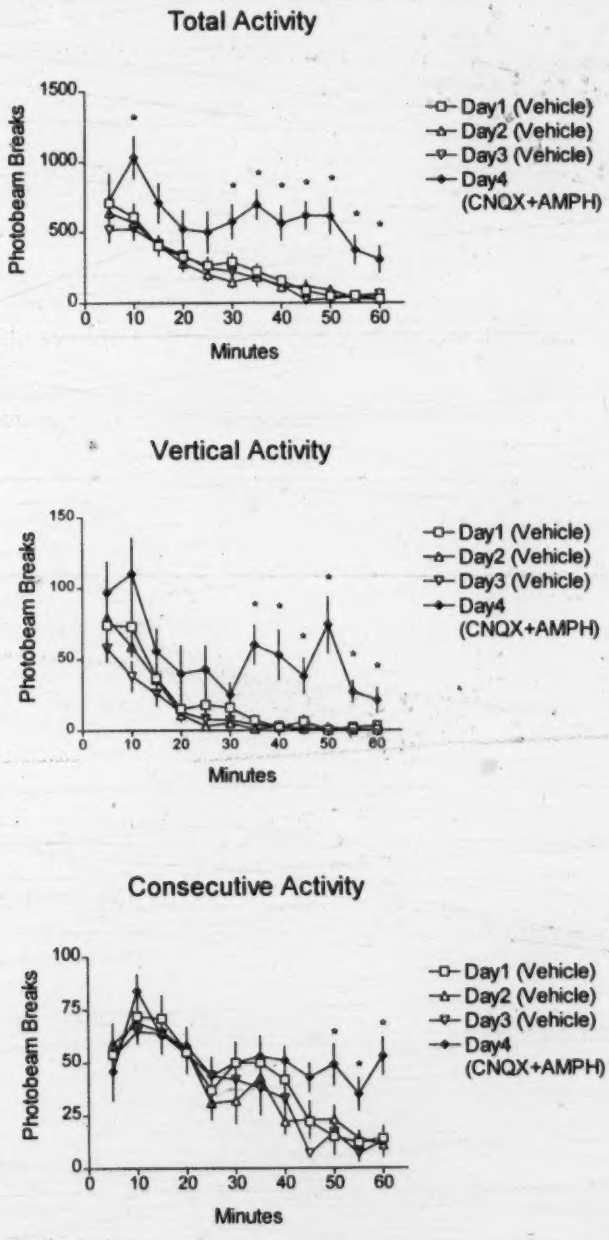


Figure 6.5. Effect of a mixture of CNQX and AMPH on repeated CNQX-treated group. Animals received three repeated microinjections of CNQX (0.5 μg) into the NAS shell (day 1-3) followed by a mixture of CNQX (0.5 μg) and AMPH (5 μg) injection on day 4. Data are means±S.E.M. (n=8) *Significant at P<0.05, relative to day 3.

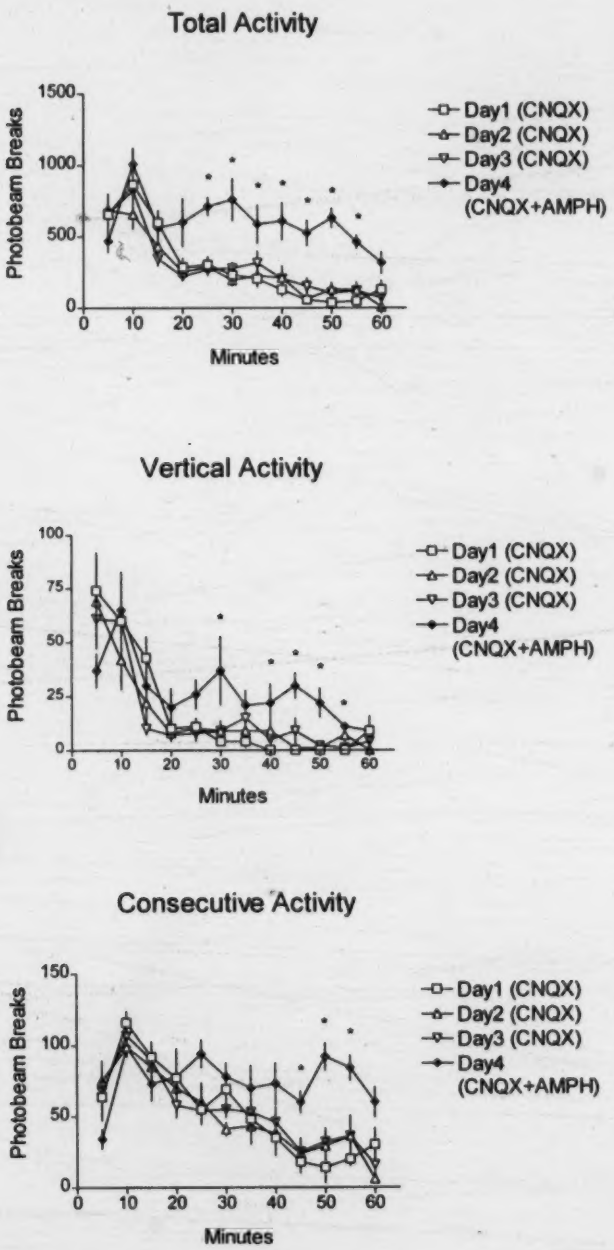


Figure 6.6. Effect of a mixture of CNQX and AMPH on repeated AMPH-treated group. Animals received three repeated microinjections of AMPH (5 μg) into the NAS shell (day 1-3) followed by a mixture of CNQX (0.5 μg) and AMPH (5 μg) injection on day 4. Data are means±S.E.M. (n=8) *Significant at P<0.05, relative to day 3.

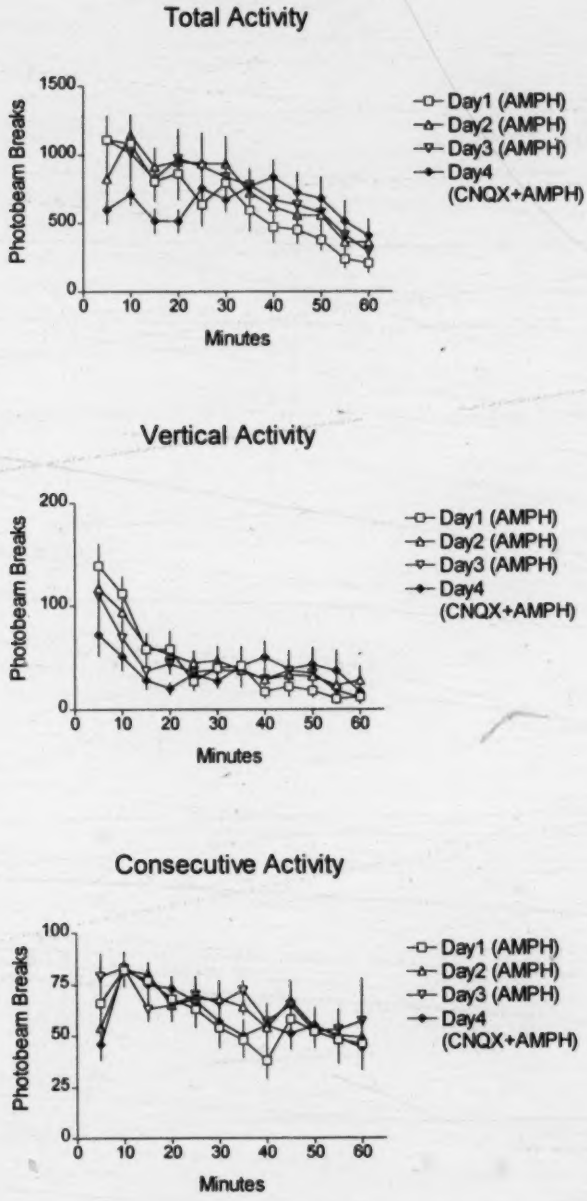
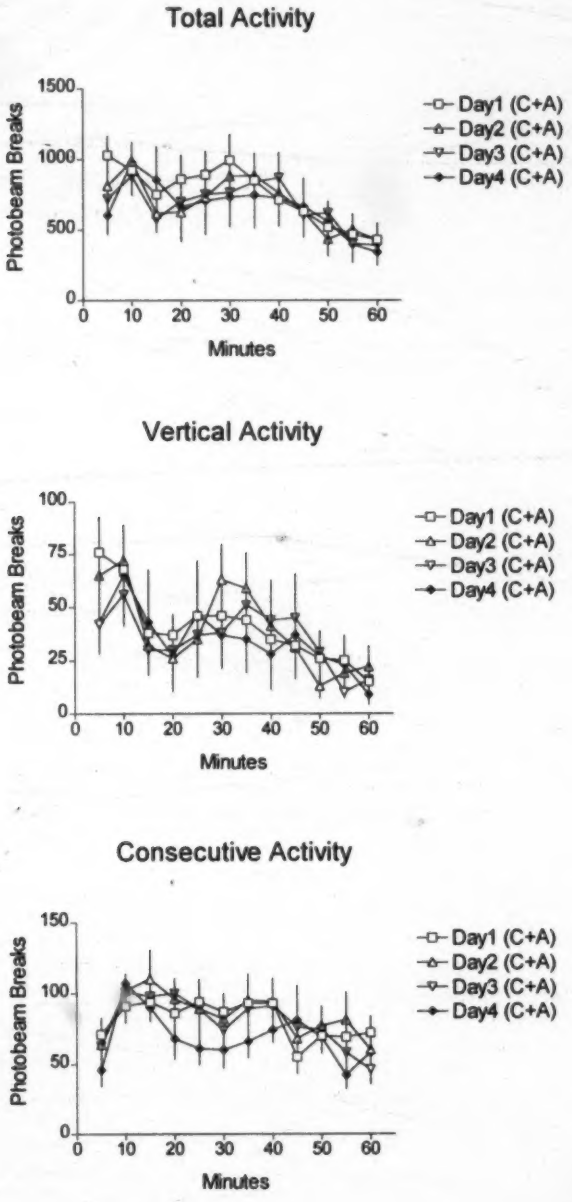


Figure 6.7. Effect of a mixture of CNQX and AMPH on repeated CNQX+AMPH-treated group. Animals received four repeated microinjections of a mixture of CNQX (0.5 μg) and AMPH (5 μg) microinjections into the NAS shell (day 1-4). Data are means±S.E.M. (n=8) *Significant at $P<0.05$, relative to day 3.



7. CNQX POTENTIATES 7-OH-DPAT-INDUCED LOCOMOTOR HYPOACTIVITY

7.1. Introduction

Interactions between dopamine and glutamate in the mesolimbic system may depend on the relative involvement of D1-like and D2-like dopamine receptors (Svensson et al. 1992a; Wu et al. 1993; Bunney 1995). Two main types of dopamine receptors have been identified as D1-like (D1 and D5) receptors and D2-like (D2, D3 and D4) receptors (Sibley et al. 1993). Although the effects of AMPA/kainate receptor antagonists on indirect dopamine receptor agonist-mediated behaviour have been reported, little is known about the effects of the D1-like and D2-like dopamine receptor agonists in the NAS sub-regions. The AMPA/kainate receptor antagonist CNQX induced a short duration of inhibitory effects on amphetamine-induced hyperactivity in the previous experiments (Chapter 5 and 6). Due to a non-selective action of amphetamine on dopamine receptors and involvement of both D1-like and D2-like receptors in locomotor activity, the effect of CNQX is difficult to interpret. Therefore, in this experiment, the effects of CNQX on direct D2/D3 dopamine receptor agonist 7-OH-DPAT in the NAS core and shell were investigated.

The D3 dopamine receptor, a member of D2-like dopamine receptor family, was identified (Sokoloff et al. 1990). This dopamine receptor received considerable interest due to its selective distribution in the mesolimbic dopamine system including the NAS,

olfactory tubercle and islands of Calleja (Daly and Waddington 1993; Diaz et al. 1995). The D3 dopamine receptor has been implicated in drug addiction and in the pathophysiology of schizophrenia (see Shafer and Levant 1998). Gurevich et al. (1997) reported that expression of D3 receptors was increased in the striatum of antipsychotic-free schizophrenic patients. However, the lack of selective compound at the D3 receptor made it difficult to study the functional role of this receptor.

An aminotetralin compound, 7-OH-DPAT has been identified as a D3-preferring agonist (Levesque et al. 1992). Recent studies which examined mammalian recombinant receptors have estimated that 7-OH-DPAT has 15 to 20-fold greater selectivity for the low-affinity states and 5 to 12-fold greater selectivity for the high affinity states of D3 receptors relative to D2 receptors (see Neve and Neve 1997). In animal studies, systemic administration of low doses of 7-OH-DPAT (<0.1 mg/kg) decreased locomotion while high doses (>0.1 mg/kg) stimulated locomotor activity (Daly and Waddington 1993; Pugsley et al. 1995). Microinjection of 7-OH-DPAT (1-3 μg) into the NAS also reduced locomotor activity (Gilbert and Cooper 1995). In agreement with these results, the D3-preferring antagonists nafadotride and U-99194A stimulated locomotor activity (Sautel et al. 1995; Waters et al. 1994). Although *in vivo* selectivity of 7-OH-DPAT for D3 receptors may be significantly lower than the results from *in vitro* ligand-binding and functional studies, this compound has been widely used as a D3-preferring agonist in behavioural studies (Levesque 1996).

The purpose of this experiment was to investigate the effect of CNQX on

locomotor activity induced by the D2/D3 dopamine receptor agonist 7-OH-DPAT in the core or shell of the NAS. Previous studies demonstrated that NMDA receptor antagonists may have differential effects on D1 and D2/D3 dopamine receptor agonist-induced behaviour (Svensson et al. 1994a; Bunney et al. 1995). For example, the NMDA receptor antagonist MK-801 enhanced the actions of the D1 dopamine receptor agonist SKF 38393 but attenuated the actions of the D2/D3 receptor agonist quinpirole in "monoamine-depleted" mice (Svensson et al. 1992a). However, no information is currently available on the effect of AMPA/kainate antagonist on locomotion induced by the actions of D2/D3 dopamine agonists in the NAS core and shell. Based on previous studies (Gilbert and Cooper 1995; Zarandi 1998), administration of 7-OH-DPAT (5 μg) into the NAS was expected to decrease locomotor activity. It was hypothesised that CNQX may inhibit 7-OH-DPAT-induced locomotor hypoactivity.

7.2. Methods

7.2.1. Microinjection of CNQX and 7-OH-DPAT into the NAS Core and Shell

Male Sprague-Dawley rats (250-300 g) were implanted with bilateral guide cannulae in the NAS core (n=8) or shell (n=12). One week after surgery, animals were handled and habituated to the photobeam activity boxes one hour daily for three days. Following the habituation period, each animal received a counterbalanced sequence of four microinjections comprised of vehicle, CNQX (0.5 μg), 7-OH-DPAT (5 μg) and a

mixture of CNQX and 7-OH-DPAT with a three-day interval between each microinjection. Following each microinjection, each animal was placed in the photobeam activity box and spontaneous locomotor activity was monitored for 60 min. The CNQX:HBC complex was dissolved in 0.45% saline, and 7-OH-DPAT was dissolved in a vehicle of 45% HBC aqueous solution.

7.2.2. Statistics

CNQX and 7-OH-DPAT data were analysed using three-way ANOVA with repeated measures (CNQX × 7-OH-DPAT × time). Local time-course data were analysed by one-way ANOVA across all treatments at each 5-min interval. A significant F-ratio ($P<0.05$) on a 5-min interval was followed by multiple comparison of means using Tukey's test.

7.3. Results

7.3.1. Effects of CNQX on 7-OH-DPAT-induced Hypoactivity

Administration of 7-OH-DPAT (5 μg) into the NAS shell decreased total activity ($P<0.05$). Co-administration of CNQX (0.5 μg) and 7-OH-DPAT (5 μg) into the NAS shell potentiated the effects of 7-OH-DPAT. As the interaction between CNQX and 7-OH-DPAT was statistically significant [$F(1,121) = 13.179$, $P<0.05$], simple main effects are not reported. *Post hoc* tests ($P<0.05$) were performed on the individual means to

compare with the controls. The local time-course data (line graph) revealed that 7-OH-DPAT decreased total activity compared to vehicle treatment during the first 25 min of the testing period. These effects were apparently increased by co-administration of CNQX in the first 20 min of the testing period (Figure 7.1).

Administration of 7-OH-DPAT (5 μg) into the NAS core decreased total activity ($P<0.05$). Co-administration of CNQX (0.5 μg) and 7-OH-DPAT (5 μg) into the NAS core potentiated the effect of 7-OH-DPAT [$F(1,77) = 10.723$, $P<0.05$]. The local time course data (line graph) revealed that 7-OH-DPAT decreased total activity during first 20 min of the testing period and this effect was apparently increased by co-administration of CNQX in the first 20 min of the testing period (Figure 7.2).

Administration of 7-OH-DPAT into the NAS shell reduced vertical activity ($P<0.05$). Co-administration of CNQX and 7-OH-DPAT into the NAS shell had a significant interaction [$F(1,121)=6.462$, $P<0.05$]. The local time course data (line graph) showed that this combination significantly decreased vertical activity in first 25 min of the testing period (Figure 7.3).

Administration of 7-OH-DPAT into the NAS core decreased vertical activity ($P<0.05$). Co-administration of CNQX and 7-OH-DPAT had a significant interaction [$F(1,77)=13.027$, $P<0.05$]. The local time-course data (line graph) revealed that this combination significantly decreased vertical activity in the first 15 min of the testing period (Figure 7.4).

Administration of 7-OH-DPAT into the NAS shell decreased consecutive activity

[F(1,11)=49.226, P<0.05]. Co-administration of CNQX and 7-OH-DPAT into the NAS shell had no interaction [F(1,121)=3.555, P>0.05]. The local time course data (line graph) showed both 7-OH-DPAT and a combination of CNQX and 7-OH-DPAT decreased activity during the first 20 and 30 min of the testing period (Figure 7.5).

Administration of 7-OH-DPAT into the NAS core decreased consecutive activity [F(1,7)=37.653, P<0.05]. Co-administration of CNQX and 7-OH-DPAT into the NAS core had no interaction [F(1,77)=2.977, P>0.05]. The local time course data (line graph) revealed that 7-OH-DPAT and a combination of CNQX and 7-OH-DPAT decreased activity during the first 25 and 40 min of the testing period (Figure 7.6).

7.4. Discussion

Administration of 7-OH-DPAT (5 μg) into the NAS core or shell decreased locomotor activity. The dose-response effects of 7-OH-DPAT (0.5-5 μg) in the NAS on locomotor activity were observed in a previous study in this laboratory (Zarandi 1998). Higher doses of 7-OH-DPAT (2.5-5 μg) reduced spontaneous locomotor activity when administered into the NAS in the study of Zarandi. This observation is consistent with the study of Gilbert and Cooper (1995), who observed a reduction in locomotion following administration of 7-OH-DPAT (1-3 μg) into the NAS. However, Meyer (1996) has reported biphasic effects of intra-NAS injections of 7-OH-DPAT, including stimulant effects at a very low (0.0001 μg) and a high dose (10 μg). Meyer's (1996) study used

Long-Evans rats, in contrast to our Sprague-Dawley rats, and the animals were not habituated to the locomotor activity test boxes in Meyer's (1996) study. Therefore, it is likely that both procedural and genetic differences account for the differences between the present results and those of Meyer (1996).

Administration of CNQX (0.5 μg) into the NAS core or shell did not alter locomotor activity. Previous studies also reported that the AMPA/kainate antagonists may be behaviourally inactive in rodents (Kaddis et al. 1993; Danysz et al. 1994; Bubser et al. 1995; Li et al. 1997). However, Wan and Swerdlow (1996) reported site-selective actions of CNQX on pre-pulse inhibition in rats. A high dose of CNQX (2.5 μg), infused into the NAS shell, disrupted prepulse inhibition while infusion into the core did not. It is possible that site-selective actions of CNQX observed in the prepulse inhibition study may be due to the high dose of CNQX (2.5 μg) used in that study. Lester et al. (1989) reported that high doses of CNQX may affect the glycine binding site of the NMDA receptors. Nevertheless, low doses of CNQX (0.25-0.5 μg) did not alter locomotor activity when administered either into the core or shell of the NAS in the previous experiment (Chapter 5).

Co-administration of CNQX (0.5 μg) into the NAS core or shell potentiated the locomotor suppressant effects of 7-OH-DPAT (5 μg). The potentiation effects suggest that concomitant stimulation of D2/D3 dopamine receptors and blockade of AMPA/kainate receptors in the NAS core and shell may have synergistic effects on locomotor behaviour. This observation is of interest because previous studies have

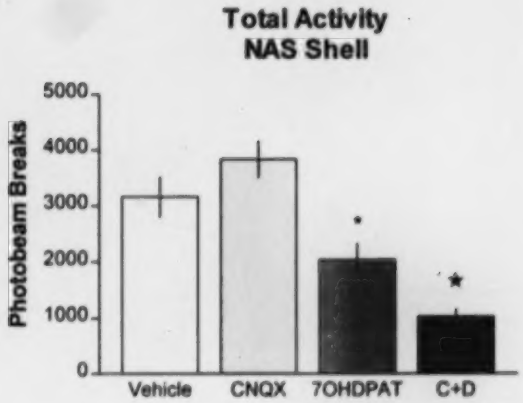
reported that the AMPA/kainate receptor antagonists attenuated locomotor hyperactivity induced by psychostimulant drugs (Kaddis et al. 1993; Burns et al. 1994). Khroyan et al. (1998) reported that 7-OH-DPAT also attenuated hyperactivity induced by amphetamine. Based on these studies, it is possible that CNQX and 7-OH-DPAT may have similar effects on amphetamine hyperactivity. Furthermore, the potentiation of 7-OH-DPAT effects by CNQX found in this experiment may be consistent with the proposal that the effect of D3 dopamine receptor stimulation is functionally opposite to the postsynaptic actions of D2 dopamine receptor agonists (Waters et al. 1993; Diaz et al. 1994; Svensson et al. 1994b).

The potentiation effect of 7-OH-DPAT by CNQX may be related to a decrease in glutamate release induced by the action of 7-OH-DPAT on glutamatergic terminals coupled with a blockade of postsynaptic AMPA/kainate receptors by CNQX. Kalivas and Duffy (1997) reported that administration of the D2/D3 dopamine agonist quinpirole into the NAS reduced extracellular glutamate levels as measured by *in vivo* microdialysis. This effect may be interpreted in terms of actions at D2/D3 dopamine receptors located on glutamate-containing terminals from cortical projections in the striatum. However, this effect was not observed following administration of the D1 dopamine agonist SKF 82958, although both D1 and D2 receptor antagonist treatment blocked reductions in glutamate induced by the indirect dopamine agonist amphetamine. With a lack of local D1 dopamine agonist action on glutamate release in this area, it is likely that CNQX may have no significant effects on D1 receptor agonist-induced behaviour (see Chapter 10).

Although CNQX has been used as an AMPA/kainate receptor antagonist, it also

has an affinity for the glycine binding site of the NMDA receptors (Lester et al. 1989). In a recent study, Mead and Stephens (1999) suggested that differential behavioural effects of CNQX and NBQX may be attributable to actions at the glycine site on the NMDA receptor and the AMPA receptor site respectively. NBQX has been identified as a more selective antagonist at the AMPA receptors compared to CNQX. Therefore, in the next experiment (Chapter 8), the effects of NBQX on hypoactivity induced by 7-OH-DPAT were examined.

Figure 7.1. Effect of CNQX in the NAS shell on 7-OH-DPAT-induced reduction in total activity. CNQX (0.5 μg) and 7-OH-DPAT (5 μg) were microinjected into the NAS shell (n=12) and total activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle. ★Significant drug × drug interaction at P<0.05.



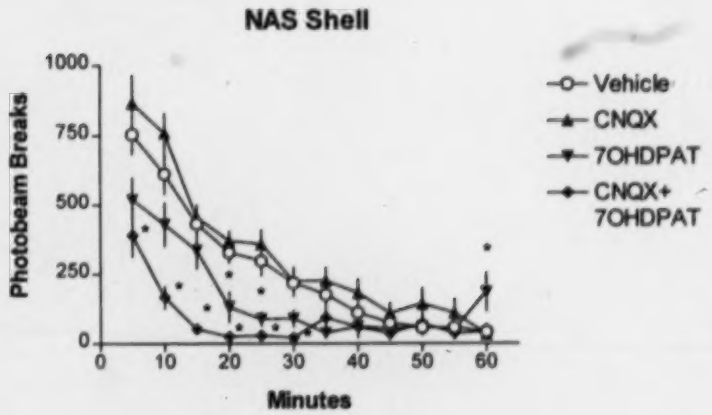
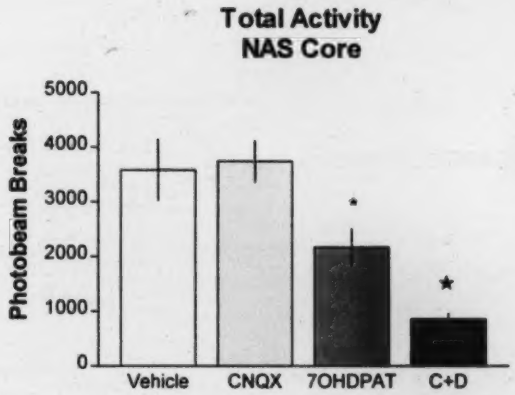


Figure 7.2. Effect of CNQX in the NAS core on 7-OH-DPAT-induced reduction in total activity. CNQX (0.5 μg) and 7-OH-DPAT (5 μg) were microinjected into the NAS core (n=8) and total activity was monitored for 60 min. Data are means±S.E.M. *Significant at $P<0.05$, relative to vehicle. ★Significant drug × drug interaction at $P<0.05$.



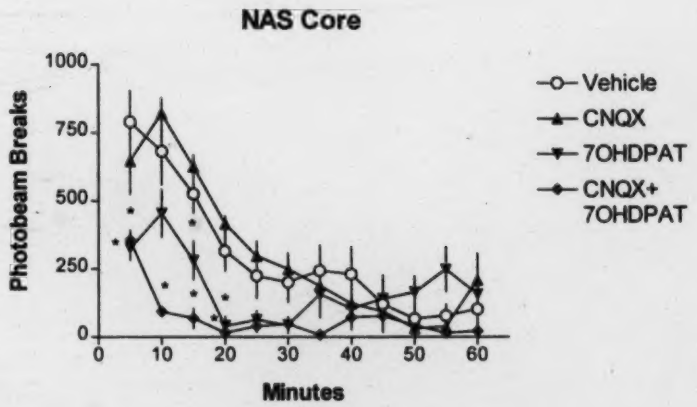


Figure 7.3. Effect of CNQX in the NAS shell on 7-OH-DPAT-induced reduction in vertical activity. CNQX (0.5 μg) and 7-OH-DPAT (5 μg) were microinjected into the NAS shell (n=12) and vertical activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle. ★Significant drug × drug interaction at P<0.05.

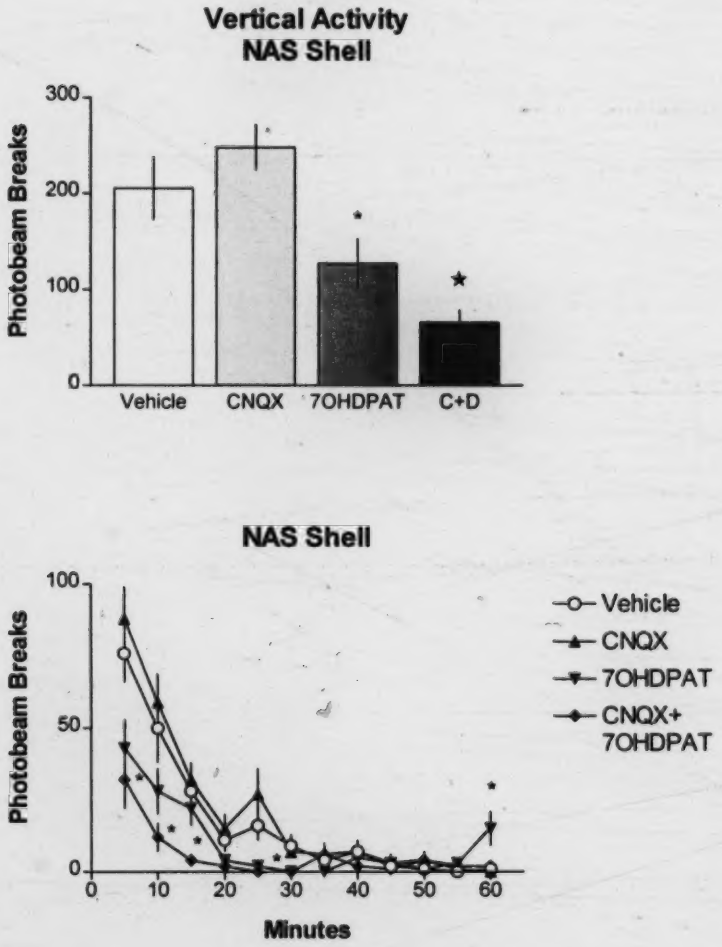
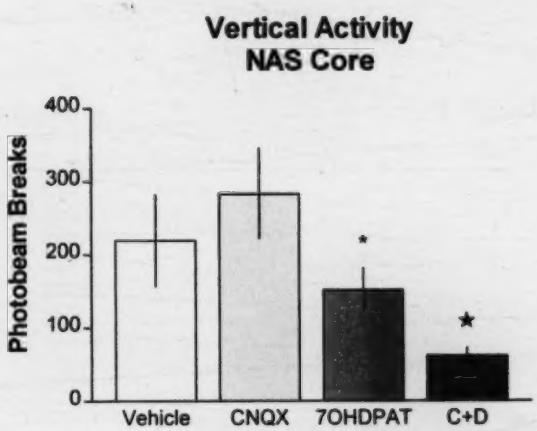


Figure 7.4. Effect of CNQX in the NAS core on 7-OH-DPAT-induced reduction in vertical activity. CNQX (0.5 μg) and 7-OH-DPAT (5 μg) were microinjected into the NAS core (n=8) and vertical activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle. ★Significant drug × drug interaction at P<0.05.



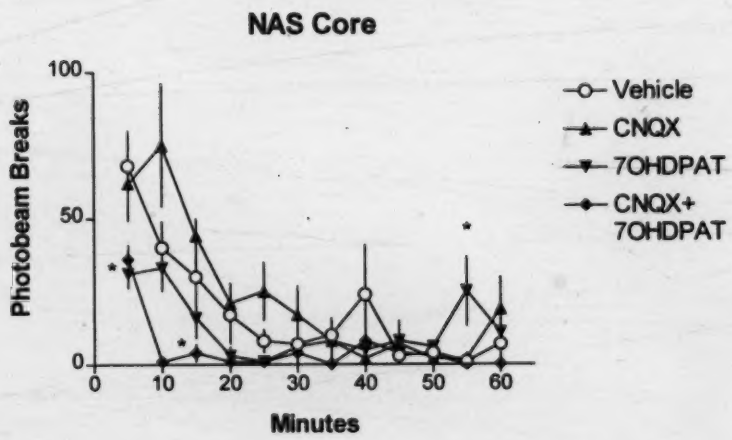


Figure 7.5. Effect of CNQX in the NAS shell on 7-OH-DPAT-induced reduction in consecutive activity. CNQX (0.5 μg) and 7-OH-DPAT (5 μg) were microinjected into the NAS shell (n=12) and consecutive activity was monitored for 60 min. Data are means± S.E.M. *Significant at P<0.05, relative to vehicle.

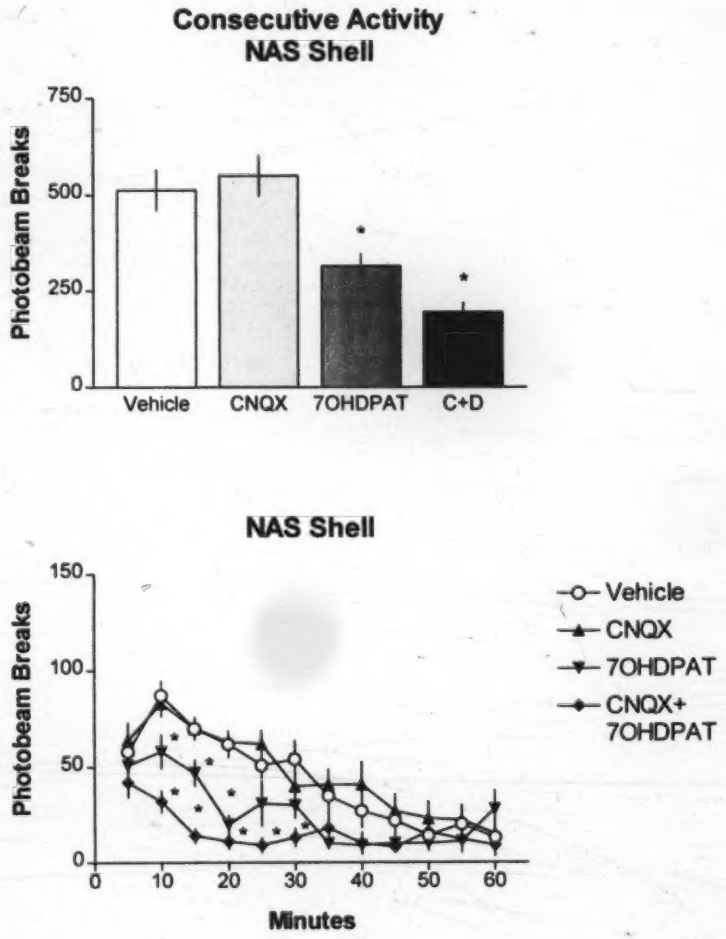
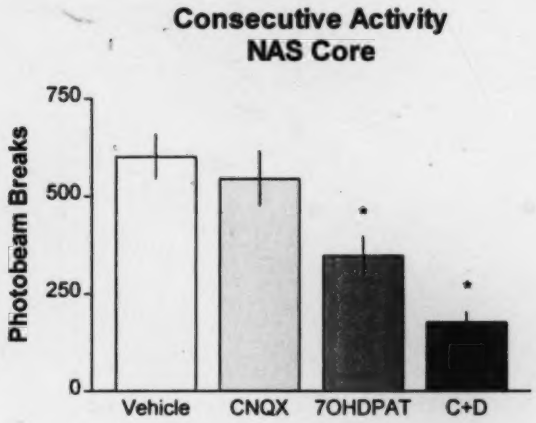
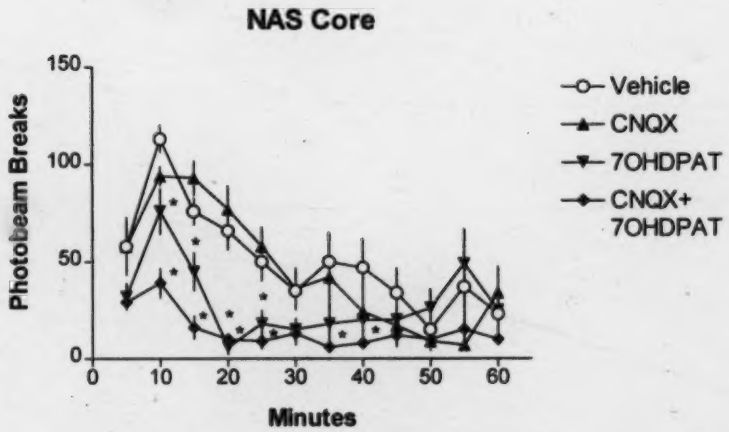


Figure 7.6. Effect of CNQX in the NAS core on 7-OH-DPAT-induced reduction in consecutive activity. CNQX (0.5 μg) and 7-OH-DPAT (5 μg) were microinjected into the NAS core (n=8) and consecutive activity was monitored for 60 min. Data are means± S.E.M. *Significant at P<0.05, relative to vehicle.





8. NBQX POTENTIATES 7-OH-DPAT-INDUCED LOCOMOTOR HYPOACTIVITY

8.1. Introduction

The synergistic effects of CNQX and 7-OH-DPAT in the NAS core and shell on locomotor activity were found in the previous experiment (Chapter 7). Although CNQX has been widely used as an AMPA/kainate receptor antagonist in previous studies, others suggested this compound has affinity for the glycine binding site of NMDA receptors at high concentrations (Birch et al. 1988; Lester et al. 1989). Previous studies suggested differential neurochemical and behavioural profiles of NMDA and AMPA/kainate antagonists (Bubser et al. 1995; Stephens and Cole 1996). Therefore the more selective AMPA receptor antagonist NBQX was used in this experiment. NBQX shows selectivity for AMPA receptors (IC_{50} vs. [^{3}H]AMPA: 0.15 μM) compared to the glycine site of the NMDA receptors (IC_{50} vs. [^{3}H]glycine: >100 μM) or the kainate receptors (IC_{50} vs. [^{3}H]kainate: 4.8 μM) (Sheardown et al. 1990).

The effects of NBQX on behaviour have been reported in previous studies. Danysz et al. (1994) reported that NBQX (30 mg/kg) induced moderate inhibitory effects on horizontal locomotor activity. Vanover (1998) reported that high doses of NBQX (10-80 mg/kg) attenuated hyperactivity and stereotypy induced by amphetamine or MK-801 in mice. This study further suggested that the AMPA receptor antagonist may attenuate the behavioural effects of amphetamine and MK-801, but not the effect of the direct

dopamine agonist apomorphine. However, Turski et al. (1992) reported that systemic administration of NBQX (16.8 mg/kg) induced muscle relaxant effects as a consequence of its effects on monosynaptic spinal reflexes. In agreement with this study, lower doses of NBQX (2.5-10 mg/kg) had no significant effect on haloperidol-induced catalepsy in rats (Zadow and Schmidt 1994). Mead and Stephens (1999) also reported a lack of effects of NBQX (10 mg/kg) on amphetamine-induced conditioned place preference, suggesting that AMPA receptor blockade may not be involved in conditioned reward mediated by amphetamine. Taken together, these results suggest that low doses of NBQX may not alter behaviour while higher doses reduce behaviour by the muscle relaxant effects of this compound. However, little is known about the central effect of NBQX on behaviour following direct administration into the brain.

The purpose of this experiment was to investigate the effects of NBQX on hypoactivity induced by 7-OH-DPAT following microinjection of these compounds into the NAS shell. The dose of NBQX (0.5 μg) used in this experiment was based on the potentiation effect of CNQX (0.5 μg) with 7-OH-DPAT (5 μg) observed in the previous experiment (Chapter 7). Based on the synergistic effect of CNQX and 7-OH-DPAT on locomotor activity, it was hypothesised that NBQX may also potentiate the effect of 7-OH-DPAT when co-administered into the NAS shell.

8.2. Methods

8.2.1. Microinjection of NBQX and 7-OH-DPAT in the NAS Shell

Male Sprague-Dawley rats (250-300 g) were implanted with bilateral guide cannulae in the NAS shell (n=9). One week after surgery, the animals were handled and habituated in the photobeam activity boxes for one hour daily for three days. Following the habituation period, each animal received a counterbalanced sequence of four microinjections comprised of vehicle, NBQX (0.5 μg), 7-OH-DPAT (5 μg) and a mixture of NBQX and 7-OH-DPAT with a three-day interval between each microinjection. Following microinjection, each animal was placed in the photobeam activity box and spontaneous locomotor activity was monitored for 60 minutes. NBQX and 7-OH-DPAT were dissolved in 45% HBC aqueous solution and 45% HBC solution was used as a vehicle.

8.2.2. Statistics

NBQX and 7-OH-DPAT data were analysed using three-way ANOVA with repeated measures (NBQX × 7-OH-DPAT × time). Local time-course data were analysed by one-way ANOVA across all treatments at each 5-min interval. A significant F-ratio ($P<0.05$) on any 5-min interval was followed by comparison of means using Tukey's test.

8.3. Results

8.3.1. Effects of NBQX on 7-OH-DPAT-induced Hypoactivity

Administration of NBQX (0.5 μg) into the NAS shell did not alter total activity. Administration of 7-OH-DPAT (5 μg) into the shell decreased total activity ($P<0.05$). Co-administration of NBQX and 7-OH-DPAT into the NAS shell potentiated the locomotor suppressant effect of 7-OH-DPAT. As the interaction between NBQX and 7-OH-DPAT was significant [$F(1,88)=5.57$, $P<0.05$], simple main effects are not reported. *Post hoc* tests ($P<0.05$) were performed to compare the individual means. The local time course data (line graph) revealed that 7-OH-DPAT and a combination of NBQX and 7-OH-DPAT reduced total activity during the first 15 min of the testing period (Figure 10.1).

Neither NBQX (0.5 μg) nor 7-OH-DPAT (5 μg) significantly altered vertical activity when administered into the NAS shell. Co-administration of NBQX and 7-OH-DPAT into the NAS shell had a significant interaction [$F(1,88)=11.508$, $P<0.05$]. The local time course data (line graph) revealed overall decreased activity with a combination of NBQX and 7-OH-DPAT, but this effect was only significant at the first 5 min of the testing period (Figure 10.2).

Administration of NBQX (0.5 μg) into the NAS shell did not alter consecutive activity [$F(1,88)=0.528$, $P>0.05$]. Administration of 7-OH-DPAT (5 μg) into the NAS shell decreased consecutive activity [$F(1,88)=55.233$, $P<0.05$]. Co-administration of NBQX and 7-OH-DPAT into the NAS shell had no interaction on consecutive activity

[F(1,88)=1.474, P>0.05]. The local time course data (line graph) revealed that a combination of NBQX and 7-OH-DPAT decreased consecutive activity during the first 25 min of the testing period (Figure 10.3).

8.4. Discussion

Administration of NBQX (0.5 μg) into the NAS shell did not significantly alter total locomotor activity, although this dose appeared to stimulate locomotor activity as revealed in the local time course analysis. It is possible that higher doses of NBQX (>0.5 μg) into the NAS shell may have stimulant effects on locomotor activity. Systemic administration of high doses of NBQX (30 mg/kg) reduced motor activity in the previous study (Danysz et al. 1994). Therefore, NBQX may induce opposite effects on locomotor activity following systemic administration or microinjection into the NAS shell. Administration of 7-OH-DPAT (5 μg) into the NAS shell decreased locomotor activity and this effect was potentiated by co-administration of NBQX (0.5 μg). The inclusion of NBQX in this experiment provides further evidence for the involvement of AMPA/kainate receptors in the interaction with 7-OH-DPAT because NBQX is a more selective AMPA/kainate receptor antagonist than CNQX. The potentiation effect of 7-OH-DPAT by NBQX confirms that simultaneous stimulation of the D2/D3 dopamine receptors and blockade of the AMPA/kainate receptors may synergistically reduce locomotor activity.

Kaddis et al. (1993) demonstrated that the AMPA/kainate receptor antagonist DNQX, when administered into the NAS, attenuated the locomotor stimulant actions of a combination of the D1 dopamine agonist (+)-SKF 38393 and the D2/D3 dopamine agonist quinpirole. However, DNQX was not tested on separate applications of (+)-SKF 38393 or quinpirole in that study. Therefore, it is not clear whether D1 or D2/D3 dopamine receptors are mainly involved in this interaction with DNQX. Based on the interaction between 7-OH-DPAT and NBQX observed in the present experiment, it is likely that the inhibitory effect of DNQX on the combination of SKF 38393 and quinpirole may have been due to the effect of DNQX on the actions of the D2/D3 receptor agonist quinpirole. However, the *in vivo* selectivities of 7-OH-DPAT and quinpirole for the D2 and D3 dopamine receptors may be different. Previous studies showed differential actions of quinpirole and 7-OH-DPAT on behaviour. Dall'Olio et al. (1997) observed that systemic administration of MK-801 reversed locomotor hypoactivity induced by a low dose of 7-OH-DPAT but not by quinpirole. These authors suggested that differential selectivities of 7-OH-DPAT and quinpirole on D3 and D2 receptors may account for this differential response to MK-801. In another study, Maj et al. (1999) also reported that the D3 receptor-preferring antagonist nafadotride counteracted locomotor activity induced by 7-OH-DPAT, but not by quinpirole. Therefore, it is possible that D2/D3 dopamine agonists 7-OH-DPAT and quinpirole may induce differential effects on behaviour.

The present results with NBQX and 7-OH-DPAT have confirmed involvement of AMPA/kainate receptors in the synergistic interaction with the D2/D3 dopamine receptor

agonist. However, *in vivo* selectivities of 7-OH-DPAT for D3 and D2 receptors have not been well characterised (see Shafer and Levant 1998). One of the limitations in studying the functional role of D3 receptors is a lack of compounds that are selective for D3 receptors. For this reason, another D2/D3 dopamine receptor agonist, (-)-quinpirole, which may have less affinity for D3 receptors than 7-OH-DPAT, was examined in the next experiment (Chapter 9).

Figure 8.1. Effect of NBQX in the NAS shell on 7-OH-DPAT-induced reduction in total activity. NBQX (0.5 μg) and 7-OH-DPAT (5 μg) were microinjected into the NAS shell (n=9) and total activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle. ★Significant drug × drug interaction at P<0.05.

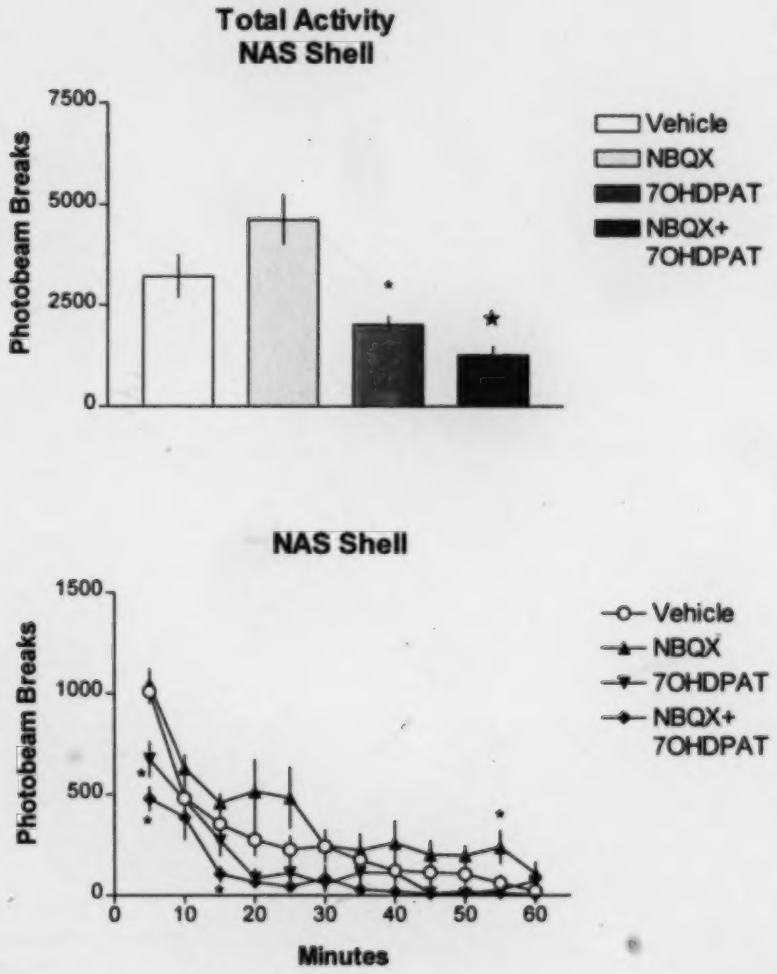


Figure 8.2. Effect of NBQX and 7-OH-DPAT in the NAS shell on vertical activity. NBQX (0.5 μg) and 7-OH-DPAT (5 μg) were microinjected into the NAS shell (n=9) and vertical activity was monitored for 60 min. Data are means±S.E.M. •Significant at P<0.05, relative to vehicle. ★Significant drug × drug interaction at P<0.05.

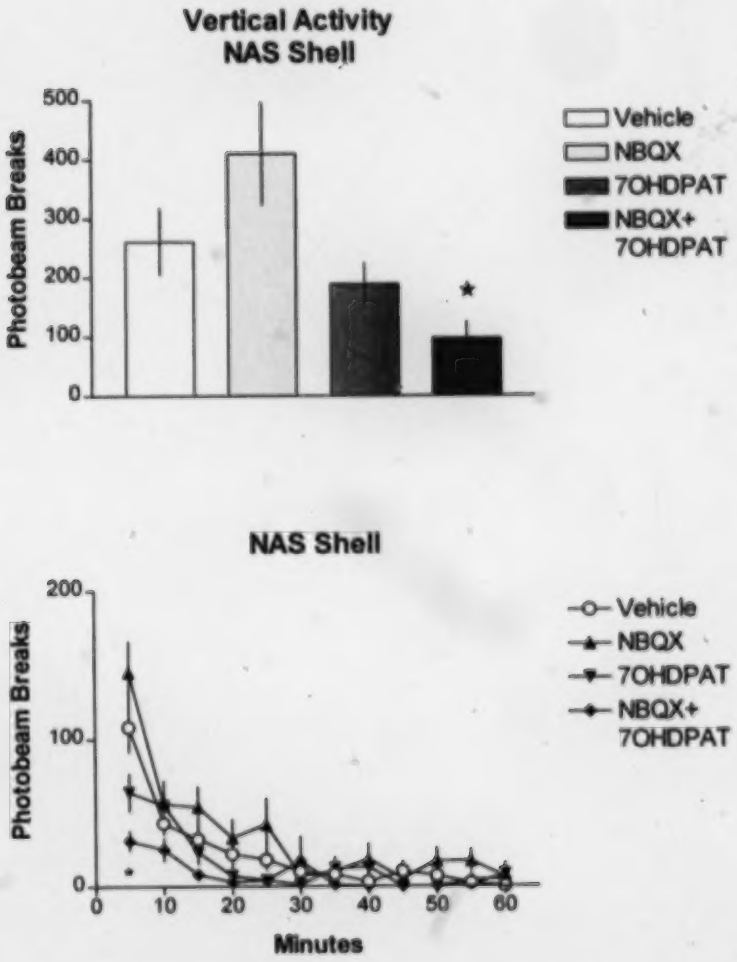
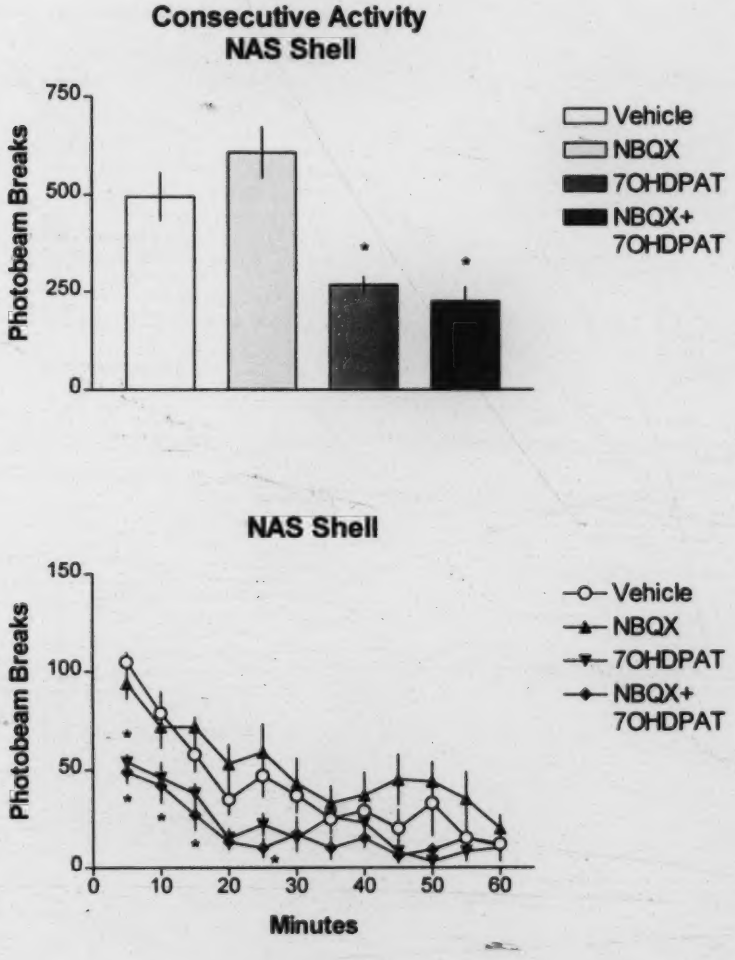


Figure 8.3. Effect of NBQX in the NAS shell on 7-OH-DPAT-induced reduction in consecutive activity. NBQX (0.5 μg) and 7-OH-DPAT (5 μg) were microinjected into the NAS shell (n=9) and consecutive activity was monitored for 60 min. Data are means± S.E.M. *Significant at P<0.05, relative to vehicle.



9. CNQX DOES NOT ALTER (-)-QUINPIROLE-INDUCED LOCOMOTOR HYPOACTIVITY

9.1. Introduction

Although synergistic effects of 7-OH-DPAT and CNQX or NBQX suggest possible involvement of D3 dopamine receptors in the interaction with the AMPA/kainate receptors, *in vivo* selectivity of 7-OH-DPAT towards the D3 receptor has been inconsistent in previous studies. The overall amino acid homology between D2 and D3 dopamine receptors is 52%, but this increases to over 70% when only transmembrane domains are considered, which indicates a structural similarity between these two subtypes of dopamine receptors (Shafer and Levant 1998). Based on studies of mammalian recombinant dopamine receptors, the D2/D3 dopamine agonist quinpirole has similar selectivity for D2 receptors but several-fold lower selectivity for D3 receptors in comparison with 7-OH-DPAT (Neve and Neve 1997).

The effects of quinpirole on locomotor activity have been reported in previous studies. Administration of quinpirole (4 μg) into the NAS decreased locomotor activity (Mogenson and Wu 1991). Canales and Iversen (1998) also reported locomotor suppressant effects of quinpirole (3 μg) when administered into the NAS. However, Van Hartesveldt et al. (1992) reported biphasic effects of quinpirole in the NAS, including stimulant effects on locomotor activity with low doses (0.1-1 μg) and suppressant effects with high doses (10-40 μg). Based on these studies, approximately 3 μg may be a

threshold dose to induce locomotor suppressant effects with quinpirole.

A recent study has reported the existence of differential effects of the NMDA receptor antagonist MK-801 on locomotor activity induced by 7-OH-DPAT and quinpirole (Dall'Olio et al. 1997). These authors suggested that MK-801 counteracted the locomotor hypoactivity induced by 7-OH-DPAT but not by quinpirole. Based on *in vitro* ligand binding studies, 7-OH-DPAT may be a more potent agonist at the D3 receptor than is quinpirole. Previous studies used 7-OH-DPAT as a D3 receptor-preferring agonist and quinpirole as a D2/D3 receptor agonist. However, relative selectivity of these compounds for the D3 receptor *in vivo* has not been well characterised. Although previous studies reported differential effects of 7-OH-DPAT and quinpirole on behaviour using systemic administration (Dall'Olio et al. 1997; Maj et al. 1999), the possible differential effects of these compounds in the NAS have not been reported.

The purpose of this experiment was to investigate the effects of CNQX on locomotor activity induced by (-)-quinpirole in the NAS core and shell. Based on the previous studies (Mogenson and Wu 1991; Swanson et al. 1997; Canales and Iversen 1998), administration of (-)-quinpirole (5 μg) into the NAS was expected to decrease locomotor activity. It was hypothesised that the less selective D3 receptor agonist (-)-quinpirole may not have a synergistic effect with CNQX when co-administered into the NAS core and shell.

9.2. Methods

9.2.1. Microinjection of CNQX and (-)-Quinpirole in the NAS Core and Shell

Male Sprague-Dawley rats (250-300 g) were implanted with bilateral guide cannulae in the NAS core (n=10) or shell (n=9). One week after surgery, the animals were handled and habituated to the photobeam activity boxes for one hour daily for three days. Following the habituation period, each animal received a counterbalanced sequence of four microinjections comprised of vehicle, CNQX (0.5 μg), (-)-quinpirole (5 μg) and a mixture of CNQX and (-)-quinpirole with a three-day interval between each microinjection. Following each microinjection, each animal was placed in the photobeam activity box and spontaneous locomotor activity was monitored for 60 min. The CNQX:HBC complex was dissolved in 0.45% saline and (-)-quinpirole was dissolved in a vehicle of 45% HBC solution.

9.2.2. Statistics

CNQX and (-)-quinpirole data were analysed using three-way ANOVA with repeated measures [CNQX × (-)-quinpirole × time]. Local time-course data were analysed using one-way ANOVA across all treatments at each 5-min interval. A significant F-ratio ($P<0.05$) on any 5-min interval was followed by comparison of means using Tukey's test.

9.3. Results

9.3.1. Effects of CNQX on (-)-Quinpirole-induced Hypoactivity

Administration of CNQX into the NAS shell did not alter total activity [$F(1,88)=0.685$, $P>0.05$]; (-)-quinpirole significantly decreased total activity [$F(1,88)=28.419$, $P<0.05$]. Co-administration of CNQX and (-)-quinpirole into the NAS shell had no interaction [$F(1,88)=0.308$, $P>0.05$]. The local time course data (line graph) revealed that (-)-quinpirole significantly reduced total activity during the first 25 min of the testing (Figure 9.1).

Administration of CNQX into the NAS core did not alter total activity [$F(1,99)=0.155$, $P>0.05$]; (-)-quinpirole significantly decreased total activity [$F(1,99)=53.799$, $P<0.05$]. Co-administration of CNQX and (-)-quinpirole into the NAS core did not result in a significant interaction [$F(1,99)=2.273$, $P>0.05$]. The local time course data (line graph) revealed that (-)-quinpirole significantly reduced total activity in the first 35 min of the testing period (Figure 9.2).

Administration of (-)-quinpirole (5 μg) into the NAS shell decreased vertical activity [$F(1,88)=9.182$, $P<0.05$]. Co-administration of CNQX and (-)-quinpirole into the NAS shell had no interaction [$F(1,88)=0.275$, $P>0.05$] (Figure 9.3).

Administration of (-)-quinpirole into the core decreased vertical activity [$F(1,99)=27.299$, $P<0.05$]. Co-administration of CNQX and (-)-quinpirole into the NAS core had no interaction [$F(1,99)=1.643$, $P>0.05$] The local time-course data (line graph)

revealed that (-)-quinpirole significantly decreased vertical activity compared to the control in the first 20 min of the testing period (Figure 9.4).

Administration of (-)-quinpirole into the NAS shell decreased consecutive activity [$F(1.88)=9.188$, $P<0.05$]. Co-administration of CNQX and (-)-quinpirole into the NAS shell resulted in no interaction with this measure [$F(1,88)=0.714$, $P>0.05$]. The local time course data (line graph) revealed that quinpirole decreased consecutive activity between 15 and 20 min of the testing period (Figure 9.5).

Administration of (-)-quinpirole into the NAS core decreased consecutive activity [$F(1,99)=42.944$, $P<0.05$]. Co-administration of CNQX and (-)-quinpirole into the NAS core did not result in a significant interaction [$F(1,99)=2.617$, $P>0.05$]. The local time course data (line graph) revealed that quinpirole decreased consecutive activity during the first 35 min of the testing period. (Figure 9.6).

9.4. Discussion

Administration of (-)-quinpirole (5 μg) into the NAS core or shell decreased spontaneous locomotor activity. This locomotor suppressant effect of (-)-quinpirole was similar to the effects of 7-OH-DPAT observed in the previous experiment (Chapter 7). Therefore, both D2 and D3 receptors in the NAS may play a role in locomotor suppressant effects induced by (-)-quinpirole and 7-OH-DPAT. Swanson et al. (1997) reported similar results with quinpirole, although the NAS core was found to be more

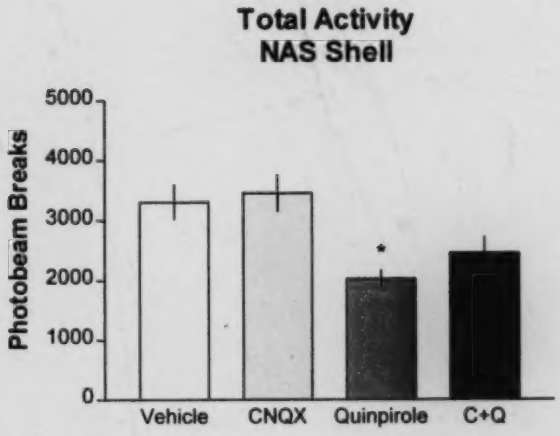
sensitive than the shell. Kaddis et al. (1993) reported that the AMPA/kainate receptor antagonist DNQX attenuated the locomotor stimulant action of a combination of SKF 38393 and quinpirole when co-administered into the NAS. In the present experiment, CNQX did not alter the effects of (-)-quinpirole when co-administered into the NAS core or shell. CNQX appeared to reverse locomotor hypoactivity induced by (-)-quinpirole when co-administered into the NAS core, but this effect was not significant in the present experiment.

Based on differential effects of 7-OH-DPAT and (-)-quinpirole in comparison with CNQX, the synergistic interaction of CNQX and 7-OH-DPAT observed in the previous experiment (Chapter 7) may have been due to relative involvement of the D3 receptors rather than the D2 receptors in the NAS. These results have important implications for understanding the mechanism of AMPA/kainate receptor blockade on the stimulant actions of psychostimulants, such as cocaine and amphetamine, on locomotor activity and other indices of behaviour (Kaddis et al. 1993; Burns et al. 1994; Wan and Swerdlow 1996).

The present results indicate that AMPA/kainate receptor blockade may have differential effects on locomotor activity depend on the relative involvement of D2 and D3 dopamine receptors in the NAS sub-regions. However, previous studies also have suggested differential effects of glutamate action on D1-like and D2-like dopamine receptors in monoamine-depleted mice (Svensson et al. 1992a; 1994a). The effects of AMPA/kainate receptor antagonists on locomotor activity induced by D1-like dopamine receptor agonists in the NAS core and shell have not been reported. Therefore, in the next

experiment (Chapter 10), the effects of CNQX on locomotor activity induced by the D1-like dopamine receptor agonist (+)-SKF 38393 in the NAS core and shell were investigated.

Figure 9.1. Effect of CNQX in the NAS shell on (-)-quinpirole-induced reduction in total activity. CNQX (0.5 μg) and (-)-quinpirole (5 μg) were microinjected into the NAS shell (n=9) and total activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle.



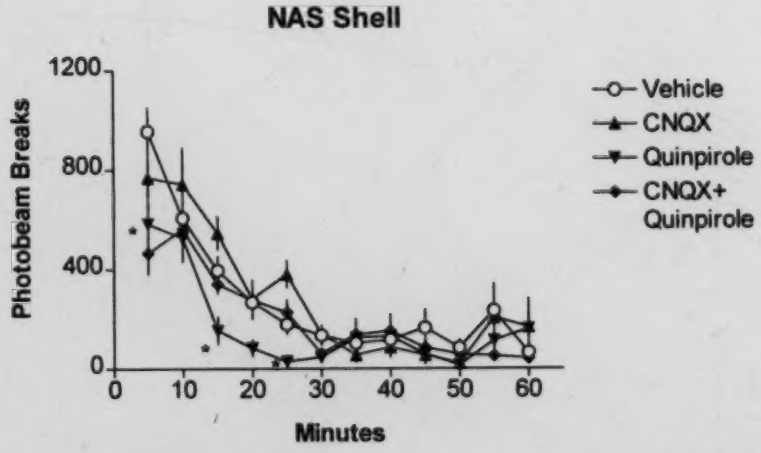
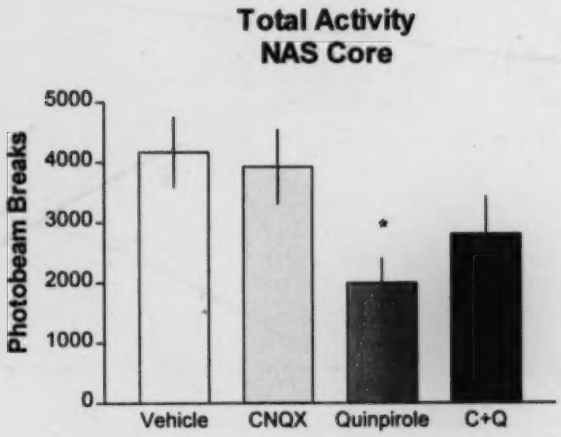


Figure 9.2. Effect of CNQX in the NAS core on (-)-quinpirole-induced reduction in total activity. CNQX (0.5 μg) and (-)-quinpirole (5 μg) were microinjected into the NAS core (n=10) and total activity was monitored for 60 min. Data are means±S.E.M. *Significant at $P<0.05$, relative to vehicle.



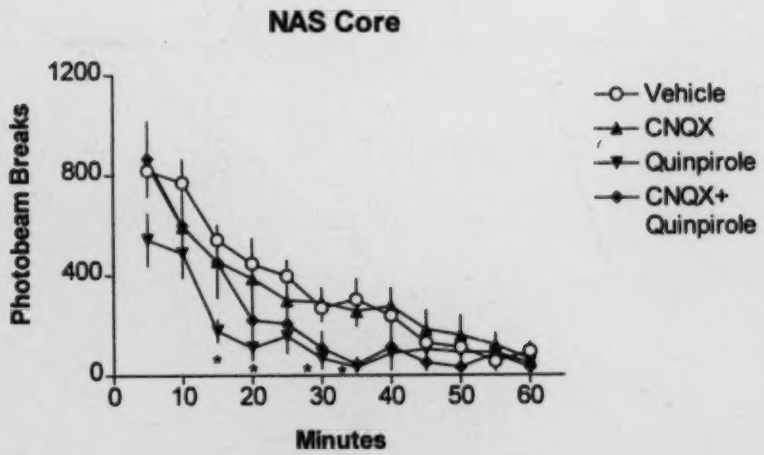
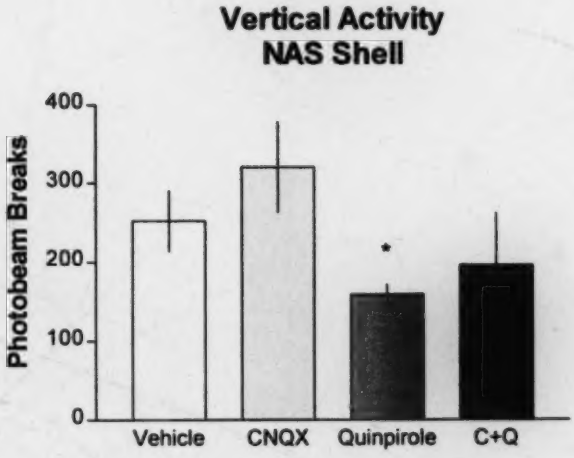


Figure 9.3. Effect of CNQX in the NAS shell on (-)-quinpirole-induced reduction in vertical activity. CNQX (0.5 μg) and (-)-quinpirole (5 μg) were microinjected into the NAS shell (n=9) and vertical activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle.



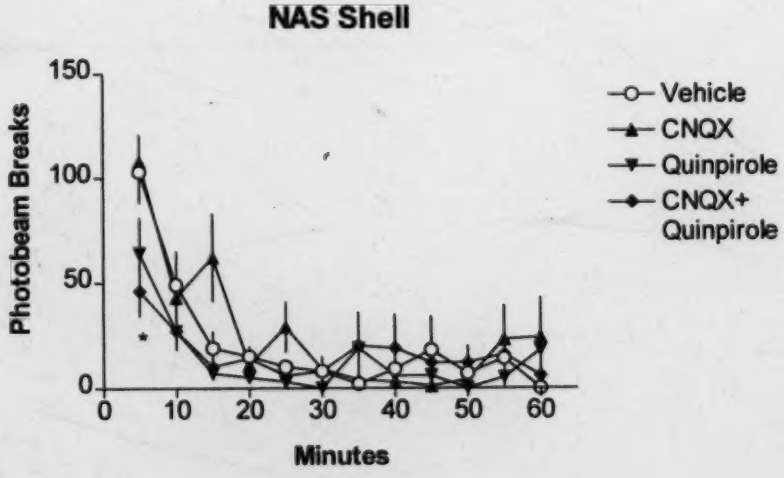
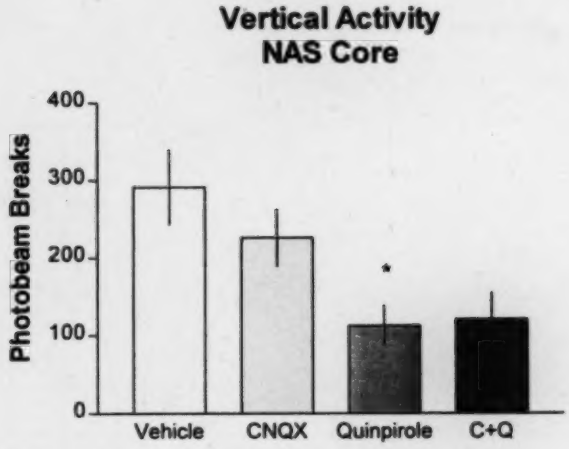


Figure 9.4. Effect of CNQX in the NAS core on (-)-quinpirole-induced reduction in vertical activity. CNQX (0.5 μg) and (-)-quinpirole (5 μg) were microinjected into the NAS core (n=10) and vertical activity was monitored for 60 min. Data are means±S.E.M. *Significant at $P<0.05$, relative to vehicle.



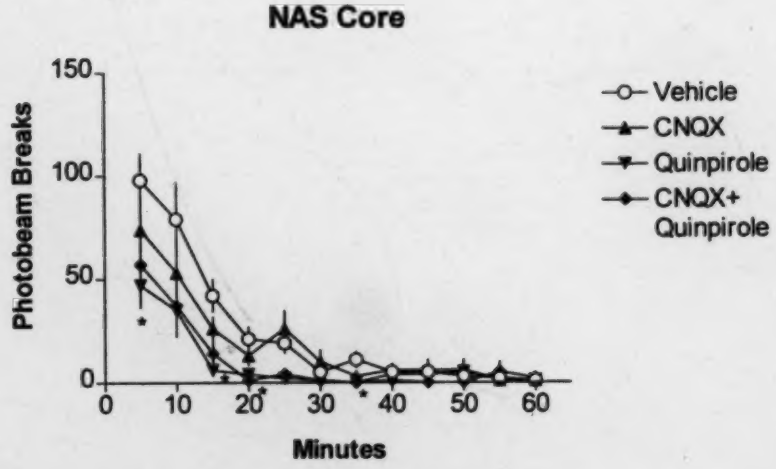
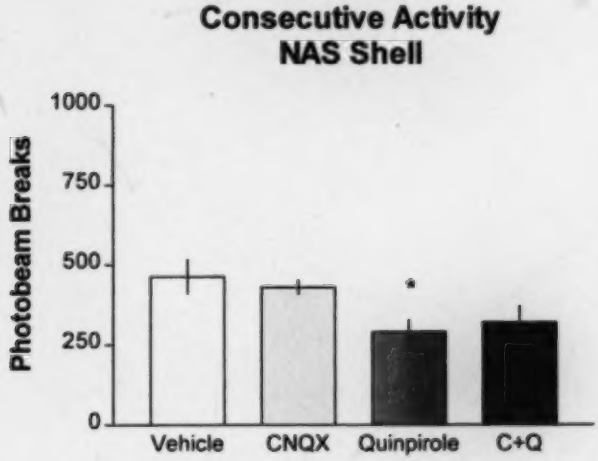


Figure 9.5. Effect of CNQX in the NAS shell on (-)-quinpirole-induced reduction in consecutive activity. CNQX (0.5 μg) and (-)-quinpirole (5 μg) were microinjected into the NAS shell (n=9) and consecutive activity was monitored for 60 min. Data are means± S.E.M. *Significant at P<0.05, relative to vehicle.



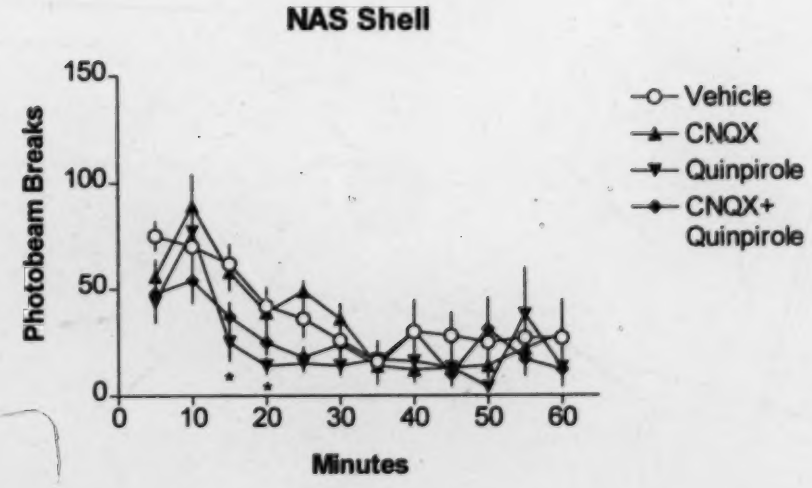
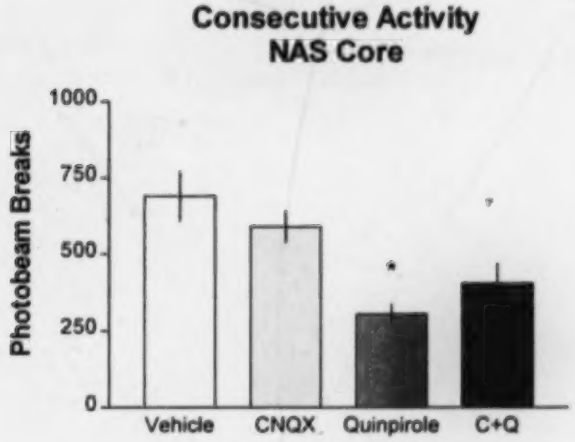
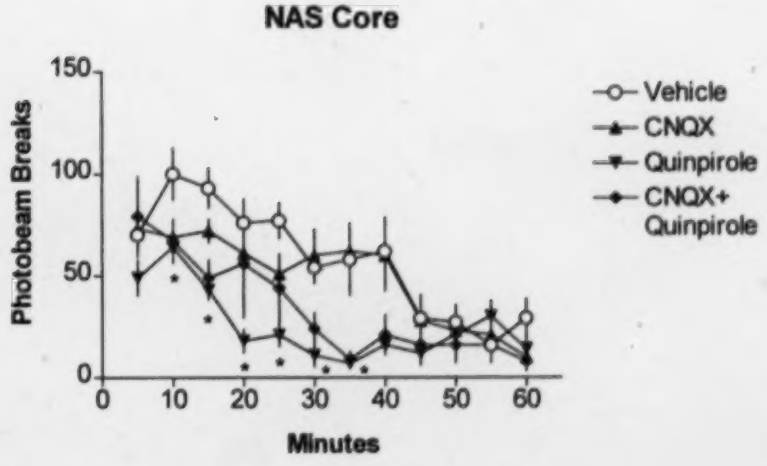


Figure 9.6. Effect of CNQX in the NAS core on (-)-quinpirole-induced reduction in consecutive activity. CNQX (0.5 μg) and (-)-quinpirole (5 μg) were microinjected into the NAS core (n=10) and consecutive activity was monitored for 60 min. Data are means ±S.E.M. *Significant at P<0.05, relative to vehicle.





10. CNQX DOES NOT ALTER (+)-SKF 38393-INDUCED LOCOMOTOR HYPERACTIVITY

10.1. Introduction

D1-like (D1 and D5) and D2-like (D2, D3 and D4) dopamine receptor agonists may induce distinct behavioural profiles in rodents (Waddington et al. 1995). The D2/D3 dopamine receptor agonists 7-OH-DPAT and (-)-quinpirole were shown to decrease locomotor activity when administered into the NAS core and shell in the previous experiments (Chapter 7 and 9). Molloy and Waddington (1987) reported that stimulation of D1-like dopamine receptors may also play a role in the regulation of locomotor activity. Systemic administration of D1-like dopamine agonists has been reported to induce a variety of behaviour such as locomotion, grooming, sniffing and vacuous chewing in rodents. A benzazepine compound, SKF 38393, which stimulates adenylyl cyclase, was identified as a D1-like agonist (Setler et al. 1978).

Interactions between dopamine and glutamate may depend on relative involvement of D1-like and D2-like dopamine receptors in the mesolimbic system (Svensson et al. 1994a; Bunney et al. 1995). Svensson et al. (1995) reported synergistic stimulant effects of the D1-like dopamine agonist SKF 38393 and the NMDA receptor antagonist MK-801 in monoamine-depleted mice. These authors suggested that the NAS is a major target site in this interaction of SKF 38393 and MK-801 using a microinjection

technique. Kaddis et al. (1993) reported interactions between AMPA/kainate receptor antagonists and dopamine receptor agonists on exploratory behaviour following microinjection into the rat NAS. Locomotor stimulant effects of a combination of the D1-like agonist SKF 38393 and the D2-like agonist quinpirole into the NAS were inhibited by the AMPA/kainate receptor antagonist DNQX in that study. However, the effect of SKF 38393 alone has not been tested with DNQX. Little information is available on the effects of AMPA/kainate receptor antagonists on behavioural responses to D1-like dopamine agonists in the NAS.

The purpose of this experiment was to investigate the effects of CNQX on locomotor activity induced by (+)-SKF 38393 in the NAS core and shell. Although previous studies examined the effects of AMPA/kainate receptor antagonists with indirect or mixed D1 and D2 dopamine receptor agonists in the NAS, the effects of D1-like receptor agonists on interaction with AMPA/kainate antagonists in the NAS core and shell have not been reported. Based on previous studies, administration of (+)-SKF 38393 into the NAS was predicted to increase locomotor activity (Dreher and Jackson 1989; Swanson et al. 1997). It was hypothesised that, if the effect of CNQX on locomotor activity induced by dopamine agonists are mediated by D2-like receptors, CNQX may not alter locomotor activity induced by (+)-SKF 38393 when co-administered into the NAS core or shell.

10.2. Methods

10.2.1. Microinjection of CNQX and (+)-SKF 38393 into the NAS Core and Shell

Male Sprague-Dawley rats (250-300 g) were implanted with bilateral guide cannulae in the NAS core (n=9) or shell (n=9). One week after surgery, animals were handled and habituated to the photobeam activity boxes for one hour daily for three days. Following the habituation procedure, each animal received a counterbalanced sequence of four microinjections comprised of vehicle, CNQX (0.5 μg), (+)-SKF 38393 (5 μg) and a mixture of CNQX and (+)-SKF 38393 with a three-day interval between each microinjection. Following each microinjection, each animal was placed in the photobeam activity box and spontaneous locomotor activity was monitored for 60 min. The CNQX:HBC complex was dissolved in 0.45% saline and (+)-SKF 38393 was dissolved in 45% HBC solution. The 45% HBC solution was used as a vehicle.

10.2.2. Statistics

CNQX and (+)-SKF 38393 data were analysed using three-way ANOVA with repeated measures [CNQX × (+)-SKF 38393 × time]. Local time-course data were analysed using one-way ANOVA across all treatments at each 5-min interval. A significant F-ratio ($P<0.05$) on any 5-min interval was followed by comparison of means using Tukey's test.

10.3. Results

10.3.1. Effects of CNQX on (+)-SKF 38393-induced Hyperactivity

Administration of (+)-SKF 38393 (5 μg) into the shell increased total activity [F (1,88)= 6.70, P<0.05]; CNQX (0.5 μg) had no significant effect [F (1,88)= 0.16, P>0.05]. Co-administration of CNQX did not alter (+)-SKF 38393-induced hyperactivity [F (1,88)= 0.04, P>0.05]. The local time-course data (line graph) showed that (+)-SKF 38393 and a combination of CNQX and (+)-SKF 38393 significantly increased total activity 35 min after microinjection (Figure 10.1).

Administration of (+)-SKF 38393 (5 μg) into the NAS core increased total activity [F (1,88)= 7.88, p<0.05]; CNQX (0.5 μg) had no significant effect [F (1,88)= 0.06, p>0.05]. Co-administration of CNQX and (+)-SKF 38393 into the NAS core did not result in any interaction [F (1,88)= 0.67, p>0.05]. *Post hoc* tests (P<0.05) on individual means at each 5 min of testing (line graph) revealed that (+)-SKF 38393 increased activity at 15 and 60 min time intervals during the testing period (Figure 10.2).

Administration of (+)-SKF 38393 into the shell did not alter vertical activity [F(1,88)=5.017, P>0.05]. Co-administration of CNQX and (+)-SKF 38393 into the NAS shell increased vertical activity, but was no interaction found [F(1,88)=1.036, P>0.05]. The local time-course data analysis revealed that the combination of CNQX and (+)-SKF 38393 significantly increased vertical activity 35 min after microinjection (Figure10.3).

Administration of (+)-SKF 38393 into the core did not alter vertical activity

[F(1,88)=4.118, P>0.05]. Co-administration of CNQX and (+)-SKF 38393 into the core also did not reveal any significant interaction [F(1,88)=2.231, P>0.05] (Figure 10.4).

Administration of (+)-SKF 38393 into the NAS shell increased consecutive activity [F(1,88)=43.946, P<0.05]. There was no interaction following co-administration of CNQX and (+)-SKF 38393 into the NAS shell [F(1,88)=0.193, P>0.05], but this combination significantly increased consecutive activity compared to the control (P<0.05). The local time-course data (line graph) revealed that (+)-SKF 38393 and a combination of CNQX and (+)-SKF 38393 significantly increased consecutive activity 40 min after microinjection (Figure 10.5).

Administration of (+)-SKF 38393 into the NAS core did not alter consecutive activity [F(1,88)=0.502, P>0.05]. Co-administration of CNQX and (+)-SKF 38393 into the NAS core also failed to reveal any interaction [F(1,88)=0.069, P>0.05] (Figure 10.6).

Histological verification of the microinjection sites in the NAS core and shell is depicted in Figure 10.7. The microinjection sites illustrated were obtained from several experiments described in Chapter 7-10. A photomicrograph of the microinjection cannulae tract and magnified views of microinjection sites are represented in Figure 10.8. No significant tissue damage was observed around the injection sites.

10.4. Discussion

Administration of (+)-SKF 38393 (5 μg) into the NAS core or shell increased spontaneous locomotor activity. This result is consistent with other studies that reported

stimulant actions of this compound following administration into the NAS (Dreher and Jackson 1989; Swanson et al. 1997). In addition, repeated administration of (+)-SKF 38393 into the NAS may result in development of behavioural sensitisation (De Vries et al. 1998; Gong et al. 1999). In the present experiment, each animal received two microinjections of (+)-SKF 38393 [(+)-SKF 38393 alone, or in combination with CNQX], and all microinjections were carried out according to a counter-balanced design to control for possible sensitisation to (+)-SKF 38393. (+)-SKF 38393 induced a delayed onset of hyperactivity 30 min after microinjection into the NAS shell.

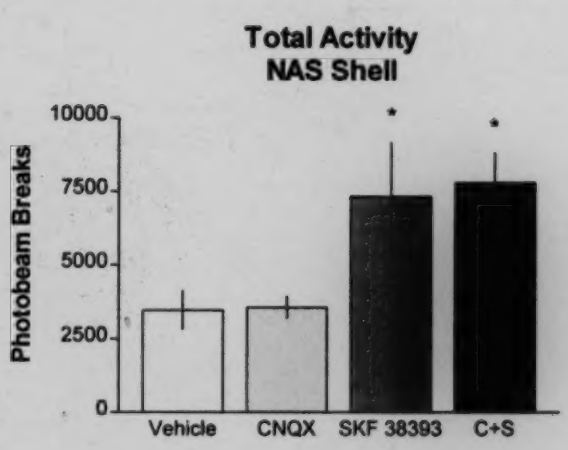
Kaddis et al. (1993) reported that administration of the structurally similar AMPA/kainate receptor antagonist DNQX into the NAS attenuated the locomotor stimulant actions of the combination of SKF 38393 and quinpirole. In the present experiment, CNQX did not alter the locomotor responses to (+)-SKF 38393 when co-administered into the NAS core or shell. However, (+)-SKF 38393 shows only partial D1 agonist activity in terms of efficacy to stimulate adenylyl cyclase and incomplete selectivity for D1 receptors vs. D5 receptors (Waddington and O'Boyle 1989). Therefore, more selective D1 receptor agonists need to be tested to resolve the possible interaction between D1 receptor agonists and AMPA/kainate receptor antagonists in the NAS core and shell.

The lack of interaction between the effects of CNQX and (+)-SKF 38393 on behaviour may be relevant to the study of Keefe and Gerfen (1999). These authors reported that infusion of CNQX did not block immediate early gene expression induced by SKF 38393 in the dopamine-depleted striatum. Therefore, it is likely that D1 receptor-

mediated responses in the dopamine-depleted striatum occur by a mechanism that is independent of glutamate input through AMPA/kainate receptors. This observation may be consistent with a lack of effect of CNQX on SKF 38393-induced locomotor hyperactivity in the present study.

Co-administration of CNQX did not alter (+)-SKF 38393-induced locomotor hyperactivity. The lack of interaction may be due to different latencies to onset of drug effect. Administration of (+)-SKF 38393 into the NAS shell induced a delayed onset of hyperactivity which lasted more than 60 min. Based on the interaction with 7-OH-DPAT (Chapter 7), it may be concluded that CNQX potentiated the effects of 7-OH-DPAT within 30 min following administration into NAS sub-regions. Therefore, co-administration of CNQX with (+)-SKF 38393 may produce immediate and delayed effects on locomotor activity. In the next experiment (Chapter 11), (+)-SKF 38393 was microinjected into the NAS shell, CNQX was microinjected 25 min after (+)-SKF 38393, and locomotor activity was monitored for a further 60 min.

Figure 10.1. Effect of CNQX in the NAS shell on (+)-SKF 38393-induced total activity. CNQX (0.5 μg) and (+)-SKF 38393 (5 μg) were microinjected into the NAS shell (n=9) and total activity was monitored for 60 min. Data are means±S.E.M. *Significant at $P<0.05$, relative to vehicle.



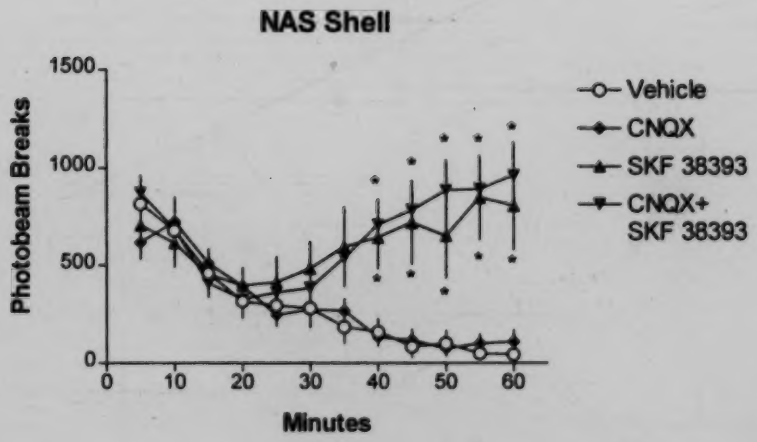


Figure 10.2. Effect of CNQX in the NAS core on (+)-SKF 38393-induced total activity. CNQX (0.5 μg) and (+)-SKF 38393 (5 μg) were microinjected into the NAS core (n=9) and total activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle.

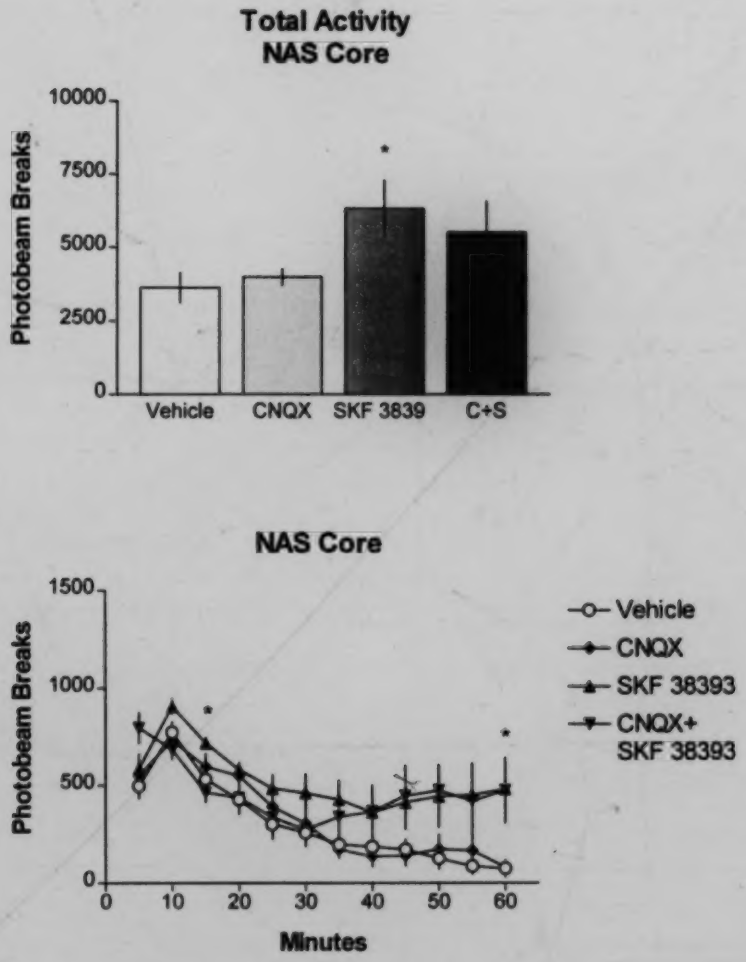
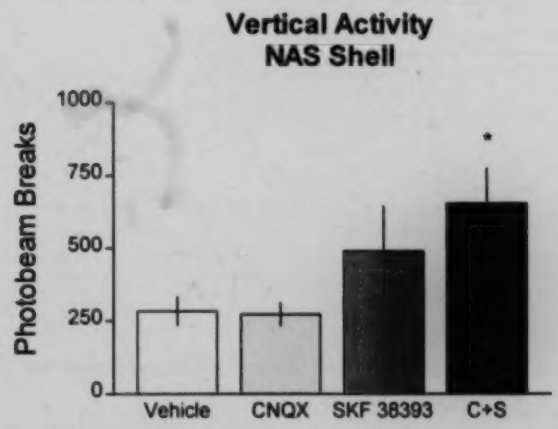


Figure 10.3. Effect of CNQX in the NAS shell on (+)-SKF 38393-induced vertical activity. CNQX (0.5 μg) and (+)-SKF 38393 (5 μg) were microinjected into the NAS shell (n=9) and vertical activity was monitored for 60 min. Data are means±S.E.M. *Significant at $P<0.05$, relative to vehicle.



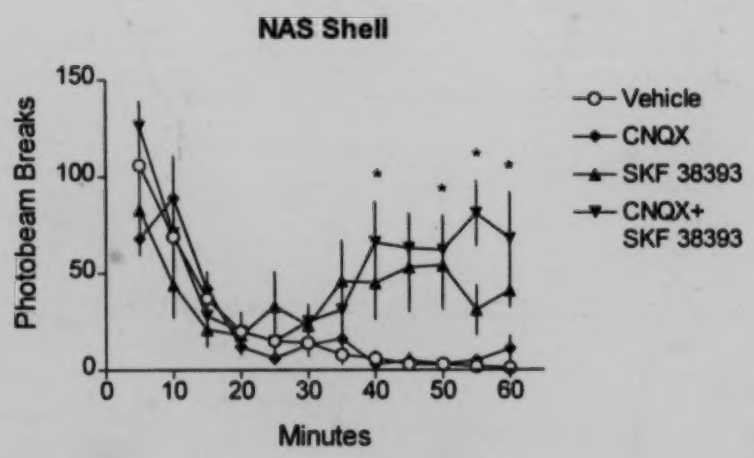
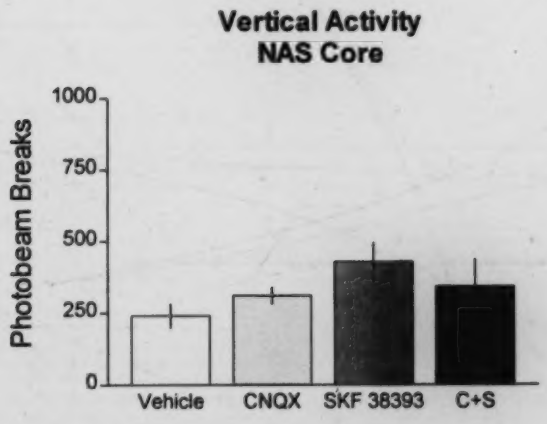


Figure 10.4. Effect of CNQX in the NAS core on (+)-SKF 38393-induced vertical activity. CNQX (0.5 μg) and (+)-SKF 38393 (5 μg) were microinjected into the NAS core (n=9) and vertical activity was monitored for 60 min. Data are means±S.E.M. *Significant at $P<0.05$, relative to vehicle.



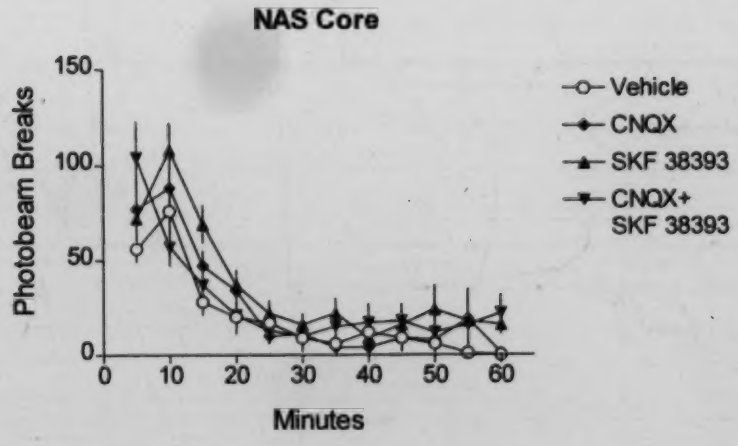
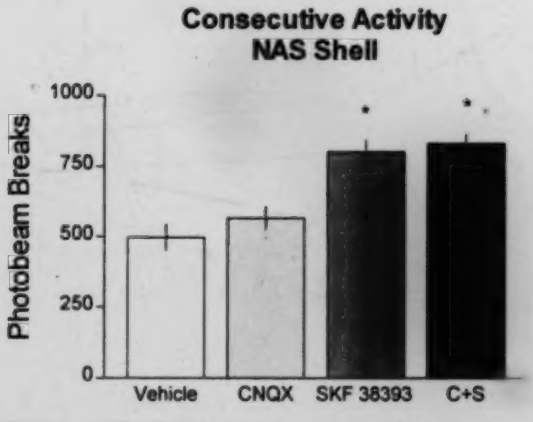


Figure 10.5. Effect of CNQX in the NAS shell on (+)-SKF 38393-induced consecutive activity. CNQX (0.5 μg) and (+)-SKF 38393 (5 μg) were microinjected into the NAS shell (n=9) and consecutive activity was monitored for 60 min. Data are means±S.E.M. *Significant at $P<0.05$, relative to vehicle.



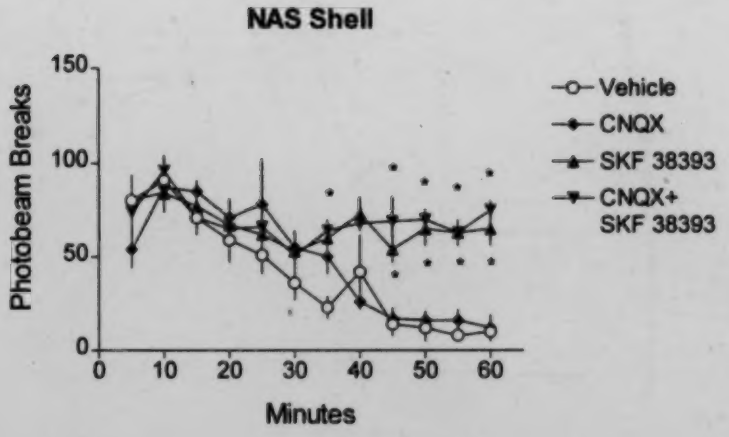
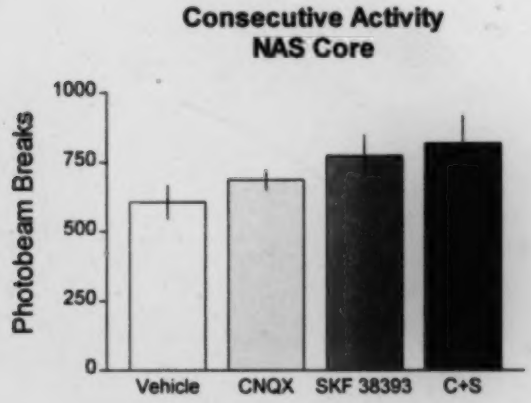


Figure 10.6. Effect of CNQX in the NAS core on (+)-SKF 38393-induced consecutive activity. CNQX (0.5 μg) and (+)-SKF 38393 (5 μg) were microinjected into the NAS core (n=9) and consecutive activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle.



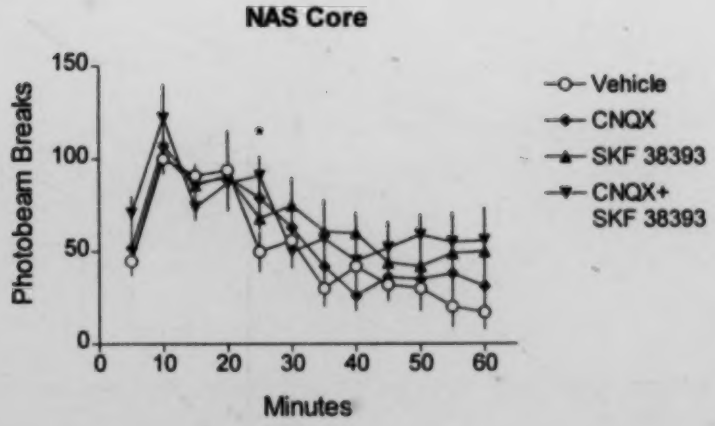


Figure 10.7. Histological verification of microinjection sites in the NAS core (•) and shell (♦). The numbers represent the distances from interaural zero according to the modified atlas of Paxinos and Watson (1986).

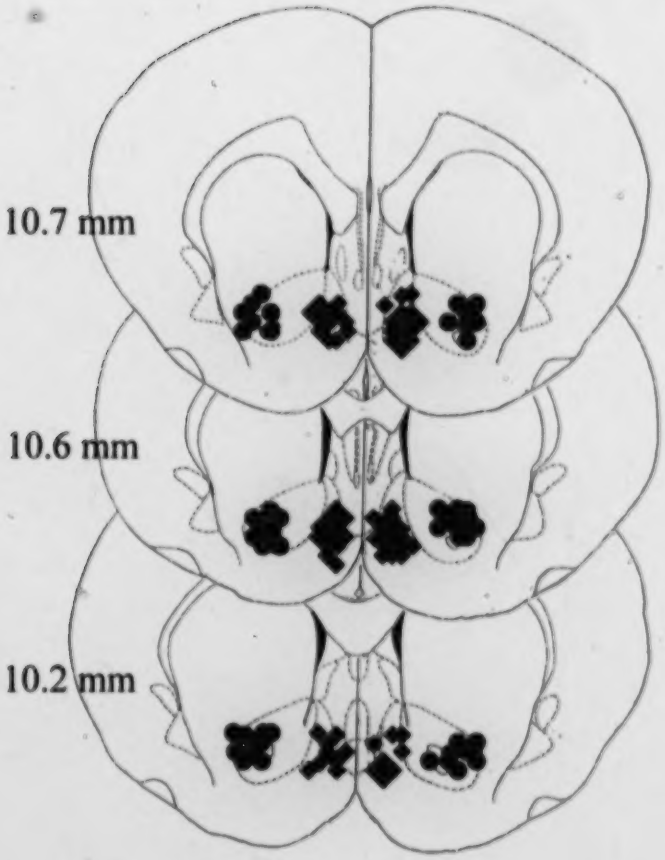
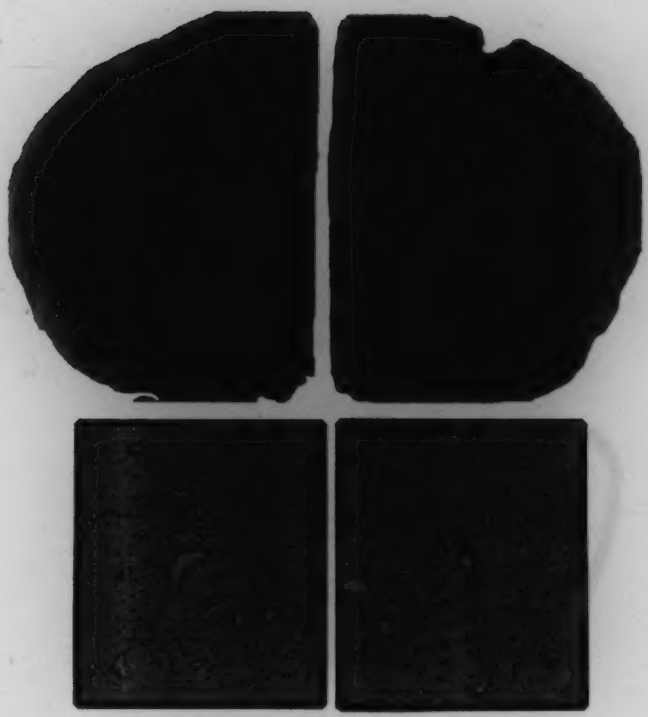


Figure 10.8. Photomicrograph of representative cannula placement in the shell (left) and core (right) of the NAS. The higher magnification in the lower panel shows the integrity of neurones following completion of microinjection and behavioural testing procedures.



11. CNQX DOES NOT ALTER LATE ONSET OF (+)-SKF 38393-INDUCED LOCOMOTOR HYPERACTIVITY

11.1. Introduction

Co-administration of CNQX and (+)-SKF 38393 into the NAS core and shell did not alter locomotor stimulant actions of (+)-SKF 38393. This observation may have been due to differential onsets of drug effects following co-administration. It has been previously shown that CNQX potentiated the locomotor suppressant effects of 7-OH-DPAT within the 30 min following co-administration into NAS (Chapter 7). In addition, (+)-SKF 38393 induced a delayed onset of locomotor hyperactivity when administered into NAS (Chapter 10). The effect of SKF 38393 is consistent with a previous study which indicates a long duration of stimulant effect following administration of SKF 38393 into the NAS (Dreher and Jackson 1989). These authors suggested that the local high concentrations of SKF 38393 achieved by intracranial microinjection produced long-lasting hyperactivity, while systemic administration did not produce this effect. Gong et al. (1999) also reported a similar delayed onset of hyperactivity following microinjection of SKF 38393 into the NAS. Therefore, due to differential onsets of drug effects, it was necessary to examine the effect of CNQX on the delayed onset of hyperactivity of (+)-SKF 38393.

The purpose of this experiment was to investigate the effects of CNQX on delayed onset of hyperactivity induced by (+)-SKF 38393 following microinjection into

the NAS shell. (+)-SKF 38393 (5 μg) was microinjected into the NAS shell, CNQX (0.5 μg) was microinjected 25 min after (+)-SKF 38393 and spontaneous locomotor activity was monitored for a further 60 min.

11.2. Methods

11.2.1. Microinjection of (+)-SKF 38393 and CNQX (25 min delay) into the NAS Shell

Male Sprague-Dawley rats (250-300 g) were implanted with bilateral guide cannulae in the NAS shell (n=12). One week after surgery, animals were handled and habituated in the photobeam activity boxes one hour daily for three days. The effects of CNQX (0.5 μg) in the NAS shell on responses to (+)-SKF 38393 (5 μg) were measured under conditions whereby CNQX was microinjected 25 min after microinjection of (+)-SKF 38393 according to a counterbalanced design. For this experiment, following the first microinjection [vehicle or (+)-SKF 38393], each animal was placed in the photobeam activity box for 25 min, removed and given the second microinjection (vehicle or CNQX) and then placed in the activity box for a further 60 min. There was a three-day interval between each microinjection. The CNQX:HBC complex was dissolved in 0.45% saline and (+)-SKF 38393 was dissolved in 45% HBC solution. The 45% HBC solution was used as a vehicle.

11.2.2. Statistics

(+)-SKF 38393 and CNQX data were analysed using three-way ANOVA with repeated measures [(+)-SKF 38393 × CNQX × time]. Local time-course data were analysed using one-way ANOVA across all treatments at each 5-min interval. A significant F-ratio ($P<0.05$) on any 5-min interval was followed by comparison of means using Tukey's test.

11.3. Results

11.3.1. Effects of CNQX on Delayed (+)-SKF 38393-induced Hyperactivity

Administration of vehicle followed by CNQX (0.5 μg) into the NAS shell did not alter total activity [$F(1,187)=0.54$, $P>0.05$]. Administration of (+)-SKF 38393 (5 μg) followed by vehicle into the NAS shell significantly increased total activity [$F(1,187)=21.92$, $P<0.05$]. There was no interaction observed after administration of (+)-SKF 38393 followed by CNQX into the NAS shell [$F(1,187)=0.67$, $P>0.05$]. The local time-course data (line graph) revealed that [(+)-SKF 38393, vehicle] and [(+)-SKF 38393, CNQX] treatment significantly increased total activity 35 min after the first microinjection, and these effects were evident for 55 min (Figure 11.1).

Administration of vehicle followed by CNQX (0.5 μg) into the NAS shell did not alter vertical activity [$F(1,187)=0.03$, $P>0.05$]. Administration of (+)-SKF 38393 (5 μg) followed by vehicle into the NAS shell did not alter vertical activity although this effect

was close to significance [F(1,187)=4.447, P=0.059]. Administration of SKF 38393 followed by CNQX into the NAS shell revealed no interaction [F(1,187)=0.346, P>0.05]. The local time course data (line graph) showed that [(+)-SKF 38393, vehicle] and [(+)-SKF 38393, CNQX] treatment significantly increased vertical activity 50 min after the first microinjection into the NAS shell, and these effects were still evident for 40 min (Figure 11.2).

Administration of CNQX (0.5 μg) followed by vehicle into the NAS shell did not alter consecutive activity [F(1,187)=0.917, P>0.05]. Administration of (+)-SKF 38393 (5 μg) followed by vehicle into the NAS shell significantly increased consecutive activity [F(1,187)=8.904, P<0.05]. Administration of (+)-SKF 38393 followed by CNQX into the NAS shell again revealed no significant interaction [F(1,187)=3.208, P>0.05]. The local time-course data (line graph) revealed that [(+)-SKF 38393, vehicle] and [(+)-SKF 38393, CNQX] significantly increased consecutive activity 50 min after the first microinjection into the NAS shell, and these effects were evident for 40 min (Figure 13.3).

11.4. Discussion

Consistent with the previous result (Chapter 10), administration of (+)-SKF 38393 into the NAS shell induced a delayed onset of hyperactivity. The locomotor stimulant effect of (+)-SKF 38393 was still prominent 90 min after microinjection, suggesting a

long duration of action of this compound in the NAS shell. Dreher and Jackson (1989) reported a similar effect of SKF 38393 on locomotor activity. CNQX did not affect delayed onset of locomotor hyperactivity when microinjected 25 min after administration of (+)-SKF 38393 into the NAS shell in the present experiment. Co-administration of CNQX and (+)-SKF 38393 into the NAS core and shell did not alter (+)-SKF 38393-induced hyperactivity in the previous experiment (Chapter 10). These results suggest that the AMPA/kainate receptor antagonist CNQX may not alter locomotor stimulation induced by the D1 receptor agonist (+)-SKF 38393. However, (+)-SKF 38393 shows only partial D1 agonist activity in terms of efficacy in stimulating adenyl cyclase and incomplete selectivity for D1 over D5 receptors (Waddington and O'Boyle 1989). It is possible that a more efficacious D1 agonist may interact with the AMPA/kainate antagonist.

The D2/D3 dopamine agonists 7-OH-DPAT and (-)-quinpirole reduced spontaneous locomotor activity while the D1 dopamine agonist (+)-SKF 38393 increased locomotor activity in the previous and present experiments. However, there was no significant difference between the NAS core and shell. Potentiation effects of CNQX on locomotor suppressant effects of 7-OH-DPAT were also observed both in the NAS core and shell. Due to the regional proximity between the NAS core and shell, it is possible that drug diffusion between these regions may affect drug action. Therefore it is necessary to study a differential drug effect in the NAS core and shell to verify the site-independent effects of CNQX and 7-OH-DPAT are not attributable to drug diffusion. Recent studies have reported that stimulation of both D1-like and D2-like dopamine

receptors in the NAS core and shell may induce differential effects on behaviour and neurochemistry (Koshikawa et al. 1996; Kitamura et al. 1999). In the next experiment (Chapter 12), the D1-like receptor agonist (+)-SKF 38393 and the D2/D3 receptor agonist (-)-quinpirole were examined to determine whether this combination had differential effects on locomotion when administered into the NAS core or shell.

Figure 11.1. Effect of CNQX in the NAS shell on delayed (+)-SKF 38393-induced total activity. (+)-SKF 38393 (5 μg) or vehicle was microinjected into the NAS shell (1st injection) followed by CNQX (0.5 μg) or vehicle microinjection (2nd injection). Total activity was monitored for a further 60 min. Data are means±S.E.M. (n=12). *Significant at $P<0.05$, relative to vehicle.

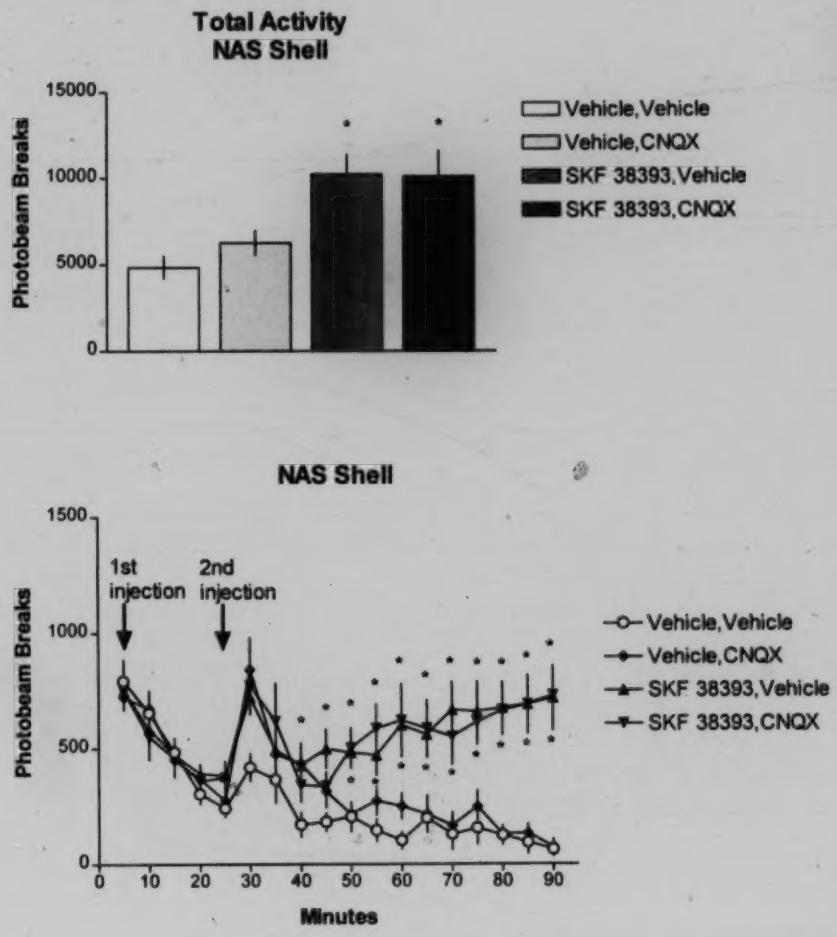


Figure 11.2. Effect of CNQX in the NAS shell on delayed (+)-SKF 38393-induced vertical activity. (+)-SKF 38393 (5 μg) or vehicle was microinjected into the NAS shell (1st injection) followed by CNQX (0.5 μg) or vehicle microinjection (2nd injection). Vertical activity was monitored for a further 60 min. Data are means±S.E.M. (n=12). *Significant at $P<0.05$, relative to vehicle.

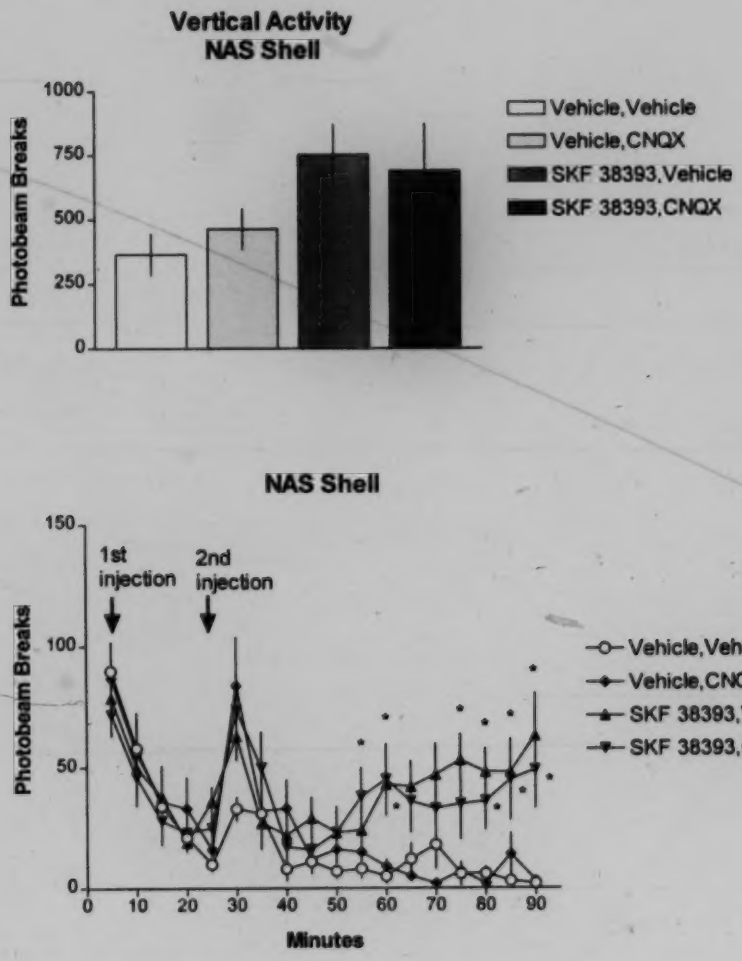
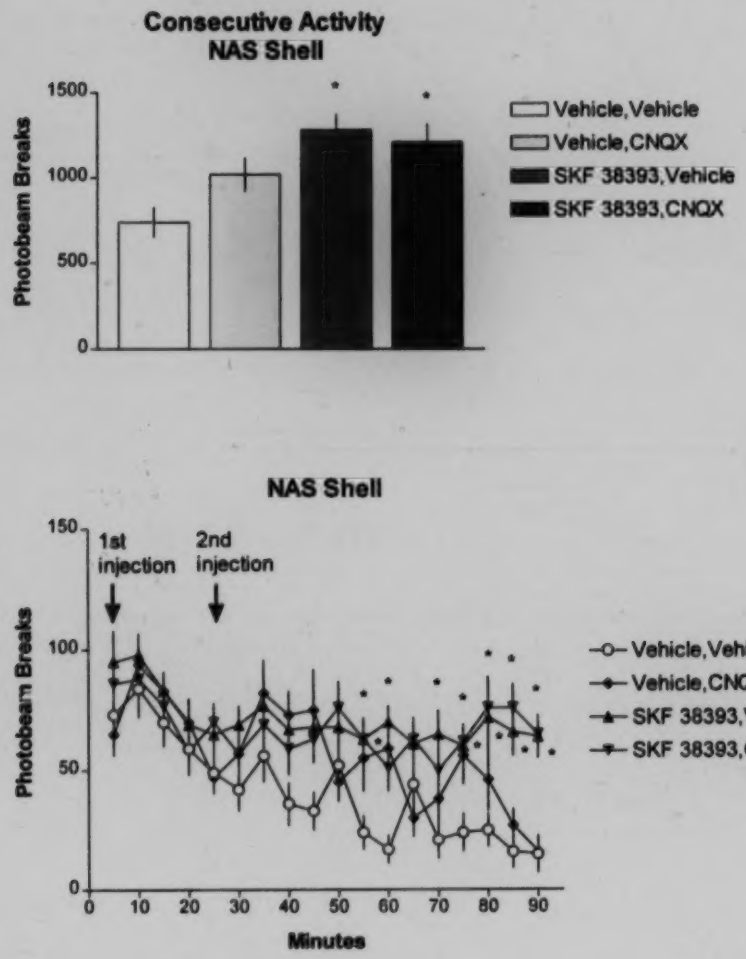


Figure 11.3. Effect of CNQX in the NAS shell on delayed (+)-SKF 38393-induced consecutive activity. (+)-SKF 38393 (5 μg) or vehicle was microinjected into the NAS shell (1st injection) followed by CNQX (0.5 μg) or vehicle microinjection (2nd injection). Consecutive activity was monitored for a further 60 min. Data are means± S.E.M. (n=12). *Significant at P<0.05, relative to vehicle.



12. (-)-QUINPIROLE POTENTIATES (+)-SKF 38393-INDUCED LOCOMOTOR HYPERACTIVITY

12.1. Introduction

An abnormal functional interaction between D1-like and D2-like dopamine receptors may play a role in the pathophysiology of schizophrenia. A link between these receptor subtypes which is mediated by GTP-binding proteins may be involved in this interaction (Seeman et al. 1989). Stimulation of both D1-like and D2-like dopamine receptors, known as the D1/D2 receptor synergism (Walters et al. 1987; Waddington et al. 1995), is normally required for dopamine function in the control of motor behaviour. For example, co-administration of D1-like and D2-like dopamine receptor agonists into the NAS synergistically increased locomotor activity in rats (Dreher and Jackson 1989; Phillips et al. 1995; Barik and Beaurepaire 1998). However, these studies did not distinguish between the core and shell of the NAS even though it is now apparent that these regions are functionally different. Recent studies suggested that co-administration of the D1-like agonist SKF 38393 (5 μg) and the D2-like agonist quinpirole (10 μg) into the NAS shell, but not the core, synergistically increased contralateral turning behaviour and jaw movement (Koshikawa et al. 1996; Kitamura et al. 1999). Furthermore, this combination of SKF 38393 and quinpirole was self-administered into the NAS shell, but not into the core, in an intracranial drug self-administration study (Ikemoto et al. 1997).

Therefore, it is likely that stimulation of D1-like and D2-like receptors in the NAS shell is necessary for this synergistic interaction. However, the synergistic effects of D1-like and D2-like dopamine receptor stimulation in the NAS core and shell on locomotor behaviour has not been well characterised.

A previous experiment (Chapter 7) demonstrated a synergistic interaction of CNQX with 7-OH-DPAT on spontaneous locomotor activity. This interaction was observed following co-administration of CNQX and 7-OH-DPAT into the NAS core and shell; there was no significant difference between these regions. However, previous studies found site-selective effects of AMPA/kainate receptor antagonist between the core and shell of the NAS on pre-pulse inhibition (Wan and Swerdlow 1996) and on feeding behaviour (Kelley 1999). Therefore, it was necessary to investigate the possible functional difference between the NAS core and shell to verify whether the site-independent effects of CNQX and 7-OH-DPAT are attributable to drug diffusion between these regions.

The purpose of this experiment was to investigate the effects of (+)-SKF 38393 and (-)-quinpirole on locomotor activity when co-administered into the core or shell of the NAS. Although previous studies reported the synergistic effect of SKF 38393 and quinpirole on locomotor activity following co-administration into the NAS, no regional analysis was reported within the NAS sub-regions. It was hypothesised that if stimulation of D1-like and D2-like receptors in the NAS shell is necessary for synergistic locomotor stimulation, co-administration of (+)-SKF 38393 and (-)-quinpirole into the NAS shell, but not the core, may synergistically increase spontaneous locomotor activity.

12.2. Methods

12.2.1. Microinjection of (-)-Quinpirole and (+)-SKF 38393 into the NAS Core and Shell

Male Sprague-Dawley rats (250-300 g) were implanted with bilateral guide cannulae in the NAS core (n=8) or shell (n=8). One week after surgery, animals were handled and habituated in the photobeam activity boxes for one hour daily for three days. Following the habituation period, each animal received a counterbalanced sequence of four microinjections comprised of vehicle, (+)-SKF 38393 (5 μg), (-)-quinpirole (2 μg) and a mixture of (+)-SKF38393 and (-)-quinpirole with a three-day interval between each microinjection. Following each microinjection, each animal was placed in the photobeam activity box and spontaneous locomotor activity was monitored for 60 min. (+)-SKF 38393 and (-)-quinpirole were dissolved in 45% HBC solution, and 45% HBC solution was used as a vehicle.

12.2.2. Statistics

(+)-SKF 38393 and (-)-quinpirole data were analysed using three-way ANOVA with repeated measures [(+)-SKF 38393 × (-)-quinpirole × time]. Local time-course data were analysed using one-way ANOVA across all treatments at each 5-min interval. A significant F-ratio ($P<0.05$) at any 5-min interval was followed by comparison of means using Tukey's test.

12.3. Results

12.3.1. Effects of (-)-Quinpirole on (+)-SKF 38393-induced Hyperactivity

Administration of (+)-SKF 38393 (5 μg) into the NAS shell increased total activity; (-)-quinpirole (2 μg) alone did not alter total activity. Co-administration of (-)-quinpirole and (+)-SKF 38393 into the NAS shell potentiated the (+)-SKF 38393 hyperactivity [$F(1,77) = 10.167$, $P<0.05$]. The local time-course data (line graph) revealed that a combination of (+)-SKF 38393 and (-)-quinpirole significantly increased total activity 20 min after microinjection into the NAS shell (Figure 12.1).

Administration of (+)-SKF 38393 (5 μg) into the NAS core increased total activity [$F(1,77)=7.331$, $P<0.05$]; (-)-quinpirole (2 μg) alone had no effect [$F(1,77)=0.590$, $P>0.05$]. Co-administration of (-)-quinpirole and (+)-SKF 38393 into the NAS core had no significant interaction [$F(1,77) = 4.159$, $P>0.05$]. The local time-course data (line graph) revealed that (+)-SKF 38393 showed overall hyperactivity, but this effect was only significant at 55 min of the testing period (Figure 12.2).

Administration of either (+)-SKF 38393 [$F(1,77)=2.511$, $P>0.05$] or (-)-quinpirole [$F(1,77)=4.801$, $P>0.05$] into the NAS shell did not alter vertical activity. Co-administration of (+)-SKF 38393 and (-)-quinpirole into the NAS shell increased vertical activity but there was no significant interaction [$F(1,77)=4.421$, $P>0.05$]. *Post-hoc* analysis ($P<0.05$) of the local time-course data showed a stimulant effect of a combination of SKF 38393 and quinpirole 25 min after microinjection (Figure 12.3).

Administration of either (+)-SKF 38393 or (-)-quinpirole into the NAS core had no significant effect on vertical activity. However, co-administration of (+)-SKF 38393 and (-)-quinpirole into the NAS core resulted in a significant interaction [$F(1,77)=8.863$, $P<0.05$], therefore simple main effects are not reported. The local time-course data (line graph) showed stimulant effects of (+)-SKF 38393 at 15 and 55 min of the testing period (Figure 12.4).

Administration of (+)-SKF 38393 into the NAS shell increased consecutive activity [$F(1,77)=16.607$, $P<0.05$]; (-)-quinpirole alone had no significant effect [$F(1,77)=0.540$, $P>0.05$]. Co-administration of (+)-SKF 38393 and (-)-quinpirole into the shell resulted in no interaction [$F(1,77)=1.475$, $P>0.05$]. The local time-course data (line graph) revealed that (+)-SKF 38393 and a combination of (+)-SKF 38393 and (-)-quinpirole significantly increased consecutive activity 30 min after microinjection. (-)-Quinpirole and a combination of (-)-quinpirole and (+)-SKF 38393 showed an inhibitory effect at 15 min of the testing period (Figure 12.5).

Administration of (+)-SKF 38393 into the NAS core did not alter consecutive activity [$F(1,77)=0.099$, $P>0.05$]; (-)-quinpirole injection into the NAS core decreased consecutive activity [$F(1,77)=44.275$, $P<0.05$]. Co-administration of (+)-SKF 38393 and (-)-quinpirole into the core revealed no interaction [$F(1,77)=2.887$, $P>0.05$]. The local time-course data (line graph) showed locomotor suppressant effects induced by (-)-quinpirole or a combination of (+)-SKF 38393 and (-)-quinpirole at 15, 20 and 30 min of the testing period (Figure 12.6).

12.4. Discussion

Administration of (+)-SKF 38393 (5 μg) into the NAS core and shell induced a delayed onset of locomotor hyperactivity. This delayed onset may be attributable to the hydrophilic properties of (+)-SKF 38393, which slowly diffuses from the injection sites and has a relatively long local retention in brain (Essman et al. 1993). Administration of (-)-quinpirole (2 μg) into the NAS core and shell did not significantly alter locomotor activity. In the previous experiment (Chapter 9), a higher dose of (-)-quinpirole (5 μg) decreased locomotor activity when administered into the NAS core or shell. These results are consistent with previous studies that reported higher doses of quinpirole (>3 μg) into the NAS may induce locomotor suppressant effects (Mogenson and Wu 1991; Van Hartesveldt et al. 1992; Canales and Iversen 1998).

(-)-Quinpirole (2 μg) potentiated the locomotor stimulant effect of (+)-SKF 38393 (5 μg) when co-administered into the NAS shell, but not into the core. There was no significant drug and drug interaction in the NAS core although this combination appeared to decrease (+)-SKF 38393-induced hyperactivity. This result needs to be explored further because vertical activity induced by (+)-SKF 38393 was reduced by (-)-quinpirole in the NAS core. The synergistic effect in the NAS shell is consistent with previous studies suggesting locomotor stimulation with D1 and D2 agonists following administration into the NAS (Dreher and Jackson 1989; Phillips et al. 1995; Gong et al. 1999). Equivalent site-selective (shell vs. core) responses to a combination of (+)-SKF

38393 (5 μg) and quinpirole (10 μg) on turning behaviour and jaw movement have recently been reported by Koshikawa et al. (1996) and Kitamura et al. (1999). These results suggest that co-activation of D1-like and D2-like receptors in the NAS shell may be necessary for locomotor stimulation in rats.

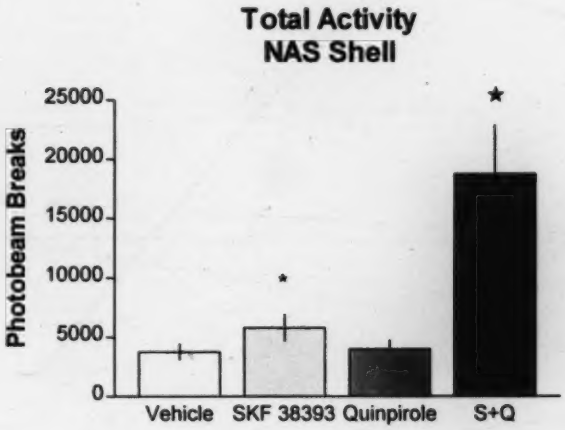
The site-selective interaction of (-)-quinpirole with (+)-SKF 38393 may be relevant to interpretation of the site-independent effects of CNQX and 7-OH-DPAT observed in the previous experiment. Based on lipophilicity, (-)-quinpirole is likely to diffuse more readily in brain than the hydroxylated compounds 7-OH-DPAT and (+)-SKF 38393. In this experiment, co-administration of (-)-quinpirole and (+)-SKF 38393 induced synergistic effects only in the NAS shell. Furthermore, a higher dose of quinpirole (10 μg) when administered with SKF 38393 (5 μg) also resulted in a site-selective response in the NAS shell (Kitamura et al. 1999). Based on these observations, the site-independent effects of 7-OH-DPAT and CNQX observed in the previous experiment (Chapter 7) are not likely due to diffusion between the NAS core and shell.

It is possible that the synergistic interaction of D1-like and D2-like dopamine receptors in the NAS shell may be mediated by different mechanisms. The D1-like and D2-like receptors may be co-localised at a single neuronal level in the NAS (Lester et al. 1993; Shetreat et al. 1996; Ridray et al. 1998). By contrast, D1-like and D2-like receptors may exist on independent neurones that interact through local synaptic GABA interneurons between distinct D1-like and D2-like receptors (Parent and Hazrati 1995a; 1995b). Furthermore, the D1-like and D2-like receptor interaction may be a result of the

direct and indirect circuitry of striatal efferents to the substantia nigra and the VTA. In this case, D1-like dopamine receptors may mediate dopamine responses to the substantia nigra by a direct pathway, while D2-like receptors may mediate the dopamine responses through the ventral pallidum *via* an indirect pathway (Gerfen 1988). Therefore, it is difficult to interpret the cellular mechanism of D1/D2 synergism observed in studies on locomotor behaviour.

Although recent studies focused on the apparent dichotomy of the NAS core vs. shell subdivisions, the functional distinction is not well understood. It has been proposed that the NAS core is more related to motor system function whereas the shell may be closely involved in motivation, related to the limbic system function (Deutch et al. 1993). However, Swanson et al. (1997) have observed that the NAS shell is more sensitive to the motor stimulant effects of dopamine agonists than the core. These authors have also demonstrated that the shell of the NAS may be preferentially involved in feeding behaviour. In addition, Carlezon and Wise (1996a, 1996b) have demonstrated that the NAS shell is an important site for mediating rewarding actions of PCP and related drugs. Therefore, in the next experiment (Chapter 13), the effects of the AMPA/kainate receptor antagonist CNQX and the D2/D3 dopamine receptor agonist 7-OH-DPAT on brain stimulation reward were investigated. CNQX and 7-OH-DPAT were co-administered into the NAS core or shell and reward threshold and response rates were measured in rats.

Figure 12.1. Effect of (-)-quinpirole in the NAS shell on (+)-SKF 38393-induced total activity. (+)-SKF 38393 (5 μg) and (-)-quinpirole (2 μg) were microinjected into the NAS shell (n=8) and total activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle. ★Significant drug × drug interaction at P<0.05.



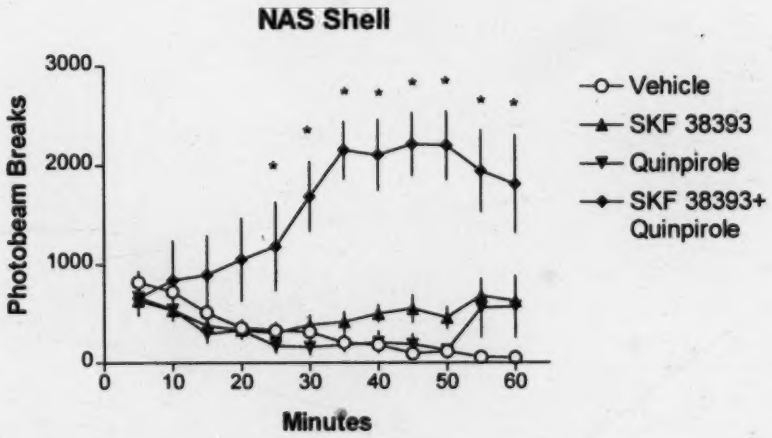
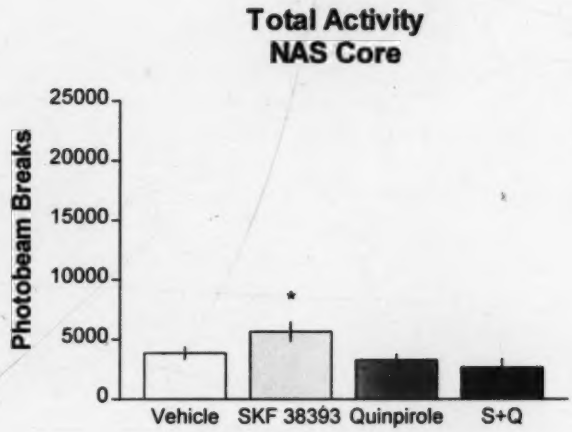


Figure 12.2. Effect of (-)-quinpirole in the NAS core on (+)-SKF 38393-induced total activity. (+)-SKF 38393 (5 μg) and (-)-quinpirole (2 μg) were microinjected into the NAS core (n=8) and total activity was monitored for 60 min. Data are means±S.E.M. *Significant at $P<0.05$, relative to vehicle.



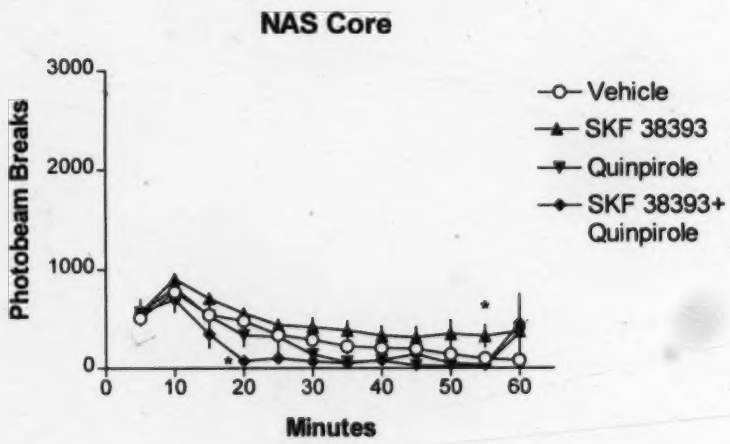
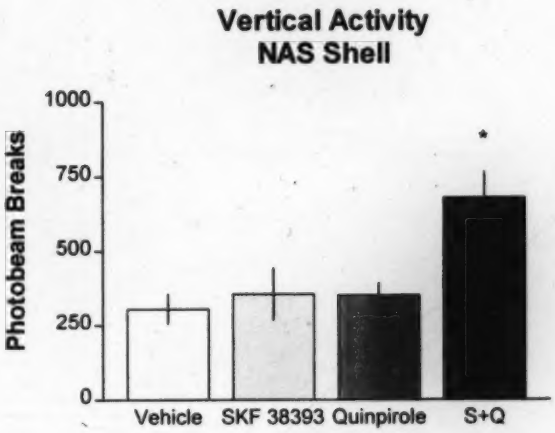


Figure 12.3. Effect of (-)-quinpirole in the NAS shell on (+)-SKF 38393-induced vertical activity. (+)-SKF 38393 (5 μg) and (-)-quinpirole (2 μg) were microinjected into the NAS shell (n=8) and vertical activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle.



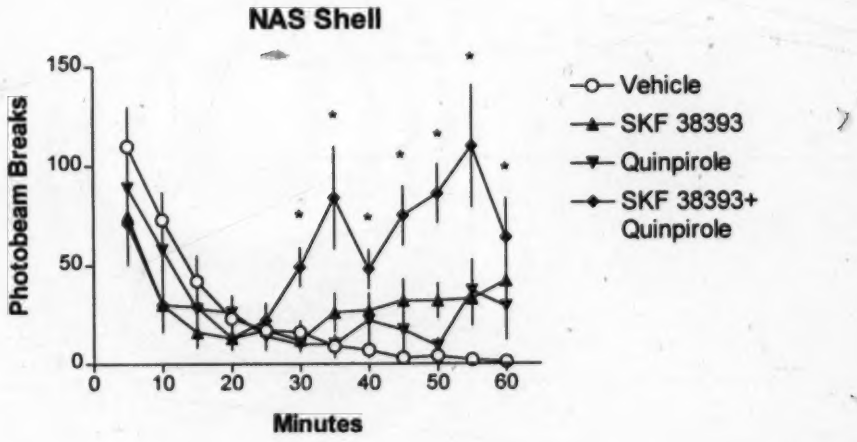
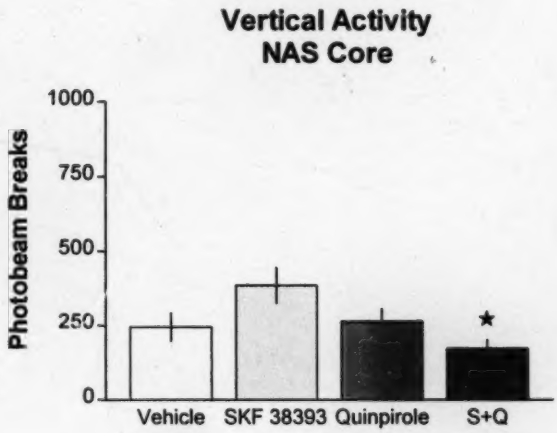


Figure 12.4. Effect of (-)-quinpirole in the NAS core on (+)-SKF 38393-induced vertical activity. (+)-SKF 38393 (5 μg) and (-)-quinpirole (2 μg) were microinjected into the NAS core (n=8) and vertical activity was monitored for 60 min. Data are means±S.E.M. *Significant at $P<0.05$, relative to vehicle. ★Significant drug × drug interaction at $P<0.05$.



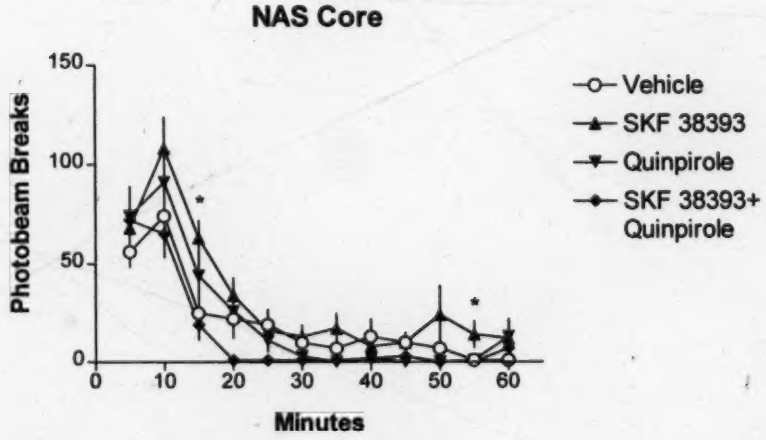
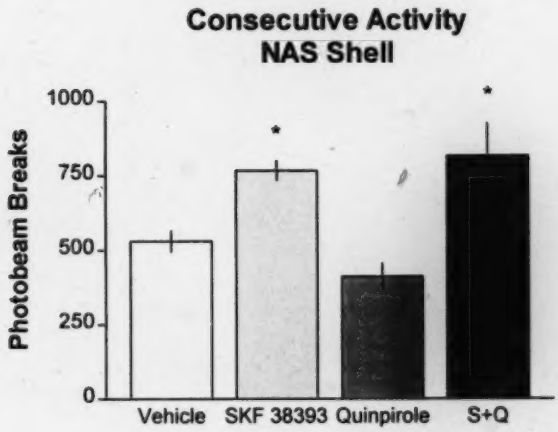


Figure 12.5. Effect of (-)-quinpirole in the NAS shell on (+)-SKF 38393-induced consecutive activity. (+)-SKF 38393 (5 μg) and (-)-quinpirole (2 μg) were microinjected into the NAS shell (n=8) and consecutive activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle.



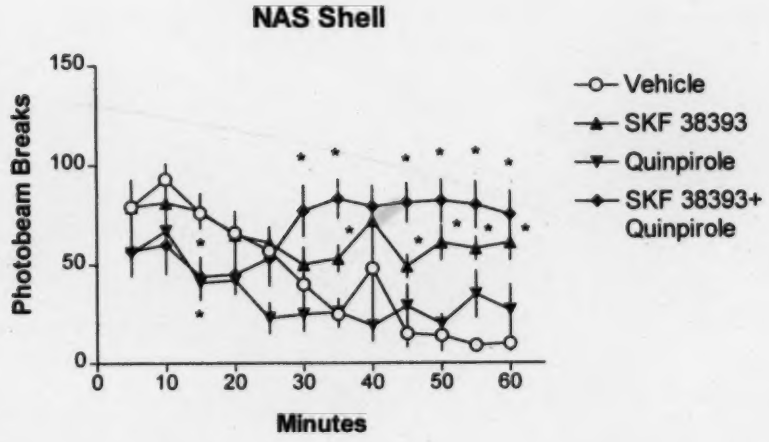
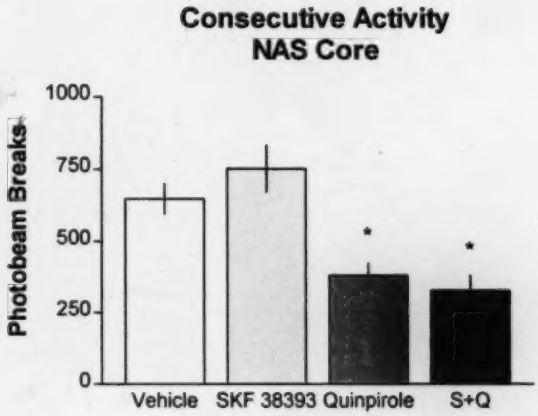
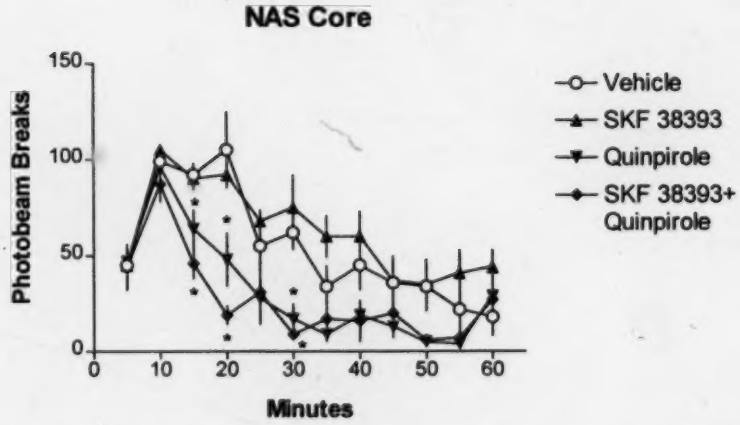


Figure 12.6. Effect of (-)-quinpirole in the NAS core on (+)-SKF 38393-induced consecutive activity. (+)-SKF 38393 (5 μg) and (-)-quinpirole (2 μg) were microinjected into the NAS core (n=8) and consecutive activity was monitored for 60 min. Data are means±S.E.M. *Significant at P<0.05, relative to vehicle.





13. A COMBINATION OF CNQX AND 7-OH-DPAT REDUCES BRAIN STIMULATION REWARD

13.1. Introduction

The interaction between dopamine and glutamate in the mesolimbic system of the brain may play a significant role in the regulation of motivated behaviour and the pathophysiology of schizophrenia (Iversen 1995; Duncan et al. 1999). The nucleus accumbens (NAS) is a major terminal area of the ascending dopamine projection from the ventral tegmental area (VTA) and descending glutamate projection from the prefrontal cortex (Sesack and Pickel, 1992; Pennartz et al. 1994). Within the NAS, the NAS shell may play a primary role in limbic functions whereas the core may be primarily involved in integrating motor system function (Deutch et al. 1993). This functional division is important because of the proposed role of the NAS in the integration and further processing of information related to emotional state and motor output (Mogenson et al. 1980; Mogenson and Yang 1991). Intracranial self-stimulation (ICSS) of the VTA may increase dopamine release in the NAS and activation of D2-like dopamine receptors in the NAS may play a role in mediating the rewarding effects of ICSS (Fibiger et al. 1987; Phillips et al. 1989; Fiorino et al. 1993). However, the effects of D2-like dopamine receptor agonists on ICSS behaviour following administration into the NAS core and shell has not been well understood.

7-OH-DPAT is a D2/D3 dopamine receptor agonist. Previous studies reported inconsistent results of 7-OH-DPAT such as decreases (Gilbert et al. 1995; Depoortere et al. 1996; 1999) or no effects (Hatcher and Hagan 1998; Baldo et al. 1999) on ICSS reward and response rate measures in rats. Gilbert et al. (1995) reported that systemic 7-OH-DPAT (0.01-0.3 mg/kg) decreased the response rate and dopamine release in the NAS following ICSS of the VTA. This study suggested that presynaptic D3 receptors in the NAS may be involved in this effect because the D2 antagonist sulpiride failed to block its dopamine-reducing effect. Depoortere et al. (1996), using a rate-frequency analysis, reported that a low dose of 7-OH-DPAT (0.1 mg/kg) induced a parallel right ward shift of the rate frequency curves, and higher doses (1-10 mg/kg) flattened the curves. These authors also found similar effects of another D2/D3 dopamine agonist, quinpirole, and a non-specific agonist, apomorphine, and suggested that these compounds decrease reward at low doses and increase reward at high doses. This group subsequently reported a decrease in the break point for the VTA ICSS on a progressive ratio schedule at doses of 0.01-0.03 mg/kg, while higher doses (1-3 mg/kg) tended to increase break point (Depoortere et al. 1999). In contrast, other studies reported that low doses of 7-OH-DPAT (0.0025-0.074 mg/kg) had no significant effects on threshold measurement of the lateral hypothalamus ICSS using a rate frequency curve-shift paradigm (Hatcher and Hagan 1998). Baldo et al. (1999) also reported that 7-OH-DPAT (0.01-1mg/kg) had no significant effects on reward threshold of the lateral hypothalamus ICSS but increased response latencies at a dose of 1mg/kg. These studies suggest that systemic administration of 7-OH-DPAT may reduce reward effects at low doses whereas high

doses may have opposite effects on reward. Although those studies reported the effects of 7-OH-DPAT on ICSS following systemic administration, no central effect of 7-OH-DPAT in the NAS core and shell has been reported.

The excitatory amino acid glutamate may also play a significant role in the rewarding effects of ICSS. Herberg and Rose (1989) reported that MK-801, a non-competitive NMDA receptor antagonist, increased response rates of lateral hypothalamus ICSS at low doses (0.01-0.1 mg/kg), but decreased these responses at higher doses (0.3 mg/kg). In that study, kynurenic acid, a nonselective glutamate receptor antagonist, produced only depression of responding, suggesting possible involvement of the non-NMDA receptors in this effect of kynurenic acid. Carlezon and Wise (1996a), using a rate-frequency analysis, reported that administration of the NMDA receptor antagonists PCP, MK-801 and CPP into the NAS shell decreased reward threshold of lateral hypothalamus ICSS. This study suggests that the NAS shell is related to the direct rewarding effects of NMDA receptor antagonists. Ranaldi et al. (1997) also reported that MK-801 facilitated the reward-enhancing effect of cocaine on lateral hypothalamus ICSS, suggesting synergistic interactions of glutamate and dopamine systems on brain stimulation reward. In contrast, Tzschentke and Schmidt (1999) found that memantine, another non-competitive NMDA antagonist, did not substantially affect the reward threshold of the lateral hypothalamus ICSS. Thus, it is likely that NMDA receptor antagonists and the AMPA/kainate receptor antagonists may have differential effects on brain stimulation reward. Previous studies have investigated the effects of NMDA receptor antagonists on ICSS, but little is known about the effects of AMPA/kainate

receptor antagonists on ICSS.

The purpose of the present study was to investigate the effects of the AMPA/kainate receptor antagonist CNQX and the D2/D3 dopamine receptor agonist 7-OH-DPAT on reward thresholds of the VTA ICSS following administration of these compounds into the NAS core and shell. Although previous studies have investigated the effects of dopamine agonists and glutamate receptor antagonists on ICSS, no regional analysis in the NAS core and shell has been reported in this context. The use of a combination of CNQX (0.5 μg) and 7-OH-DPAT (5 μg) was based on a previous study in this laboratory indicating a synergistic interaction of CNQX and 7-OH-DPAT in the NAS (Choi et al. 2000). Based on other studies, it was hypothesized that 7-OH-DPAT may increase the reward threshold and CNQX may potentiate the effect of 7-OH-DPAT. Within the NAS, the shell was predicted to be more sensitive to reward responses to these compounds on the VTA ICSS (Carlezon and Wise 1996a).

13.2. Methods

13.2.1. Microinjection of CNQX and 7-OH-DPAT into the NAS Core or Shell, and ICSS

Male Sprague-Dawley rats (300-400 g) were implanted with a monopolar nichrome electrode directed to the VTA and bilateral guide cannulae directed to the NAS core or shell. Beginning 1 week after surgery, each animal was trained to respond on the

lever, resulting in delivery of the VTA stimulation consisting of 1-sec trains of cathodal 0.2-ms pulses at a varying range of frequencies (160-10 Hz). Reward threshold and rate responses were measured daily: the frequency that maintained half-maximal response rates (M50), the maximal number of responses at a single frequency (RMAX), the total number of responses per session (TRES) and the frequency at which response rates dropped to zero (ZFRE) (see Chapter 2.4. for details).

In a factorial design, two groups of rats (core: n=6, shell: n=7) received a counterbalanced sequence of four microinjections comprised of vehicle (aCSF, pH 7.2), CNQX (0.5 μg), 7-OH-DPAT (5 μg) and a mixture of CNQX and 7-OH-DPAT. Each animal was microinjected four times with a three-day interval between each microinjection. Averaged responses during drug-free days were used as a baseline for each microinjection day response. Following each set of microinjections, each animal was placed in an operant test chamber and ICSS responses were measured. The CNQX:HBC complex was dissolved in 0.45% saline and 7-OH-DPAT was dissolved in 45% HBC solution.

13.2.2. Statistics

Effects of CNQX and 7-OH-DPAT were assessed by Friedman's two way ANOVA followed by Wilcoxon's matched-pairs signed ranks test to compare effects of drug treatment vs. control ($P<0.05$).

13.3. Results

13.3.1. Effects of CNQX and 7-OH-DPAT on ICSS

The effects of administration of CNQX (0.5 μg) and 7-OH-DPAT (5 μg) into the NAS shell (Figure 13.1) or the core (Figure 13.2) are represented by rate-frequency curves and non-linear regression curves. Rate-frequency curves were derived by converting each animal's response rate per frequency to a percentage of maximal rates and averaging the percentages for each frequency across the treatment. Non-linear regression curves calculated from the rate-frequency curves revealed that co-administration of CNQX and 7-OH-DPAT into the NAS shell or core shifted the curves rightward relative to vehicle treatment.

Neither CNQX (0.5 μg) nor 7-OH-DPAT (5 μg) had significant effects on reward thresholds (M50) when administered into the NAS shell or core. However, co-administration of CNQX and 7-OH-DPAT into the NAS shell or core significantly increased reward thresholds [shell: $\chi_r^2(3)=8.25$, $P<0.05$; core: $\chi_r^2(3)=8.8$, $P<0.05$] (Figure 13.3).

Administration of 7-OH-DPAT (5 μg) into the NAS core decreased the maximal number of responses (RMAX) [RMAX: $\chi_r^2(3)=8.492$, $P<0.05$]. Co-administration of CNQX and 7-OH-DPAT into the shell or core significantly decreased RMAX [shell: $\chi_r^2(3)=10.853$, $P<0.05$; core: $\chi_r^2(3)=8.492$, $P<0.05$] (Figure 13.4).

Administration of 7-OH-DPAT (5 μg) into the NAS core decreased the total

number of responses (TRES) [TRES: χ_r^2 (3)=9.8, P<0.05]. Co-administration of CNQX and 7-OH-DPAT into the NAS shell or core significantly decreased the total number of responses [shell: χ_r^2 (3)=7.783, P<0.05; core: χ_r^2 (3)=9.8, P<0.05] (Figure 13.5).

Neither CNQX (0.5 μg) nor 7-OH-DPAT (5 μg) when administered into the NAS shell or the core altered the zero frequency (ZFRE). Co-administration of CNQX and 7-OH-DPAT into the NAS core or shell increased ZFRE [shell: χ_r^2 (3)=9.0, P<0.05; core: χ_r^2 (3)=9.0, P<0.05] (Figure 13.6).

Histological verification of microinjection sites in the NAS core and shell and the electrode placements in the VTA are presented in Figure 13.7 and 13.8, respectively. Only animals with correct NAS core or shell and VTA placements were included in the data analysis. No significant tissue damage was observed around the microinjection sites at the end of the experiments.

13.4. Discussion

Administration of the D2/D3 dopamine receptor agonist 7-OH-DPAT (5 μg) into the NAS core or shell did not alter the reward threshold. This result is consistent with a lack of reward effect of this compound on ICSS (Hatcher and Hagan 1998; Baldo et al. 1999). It has been also reported that intra-NAS administration of the D3 receptor-preferring antagonist (+)-UH 232 did not alter reward thresholds for medial forebrain bundle self-stimulation (Nakajima and Patterson 1997). In contrast, Singh et al. (1997)

reported that administration of quinpirole (10 μg) into the NAS increased the reward threshold of VTA ICSS. However, this result may have been affected by a motor effect because this high dose of quinpirole (10 μg) has been reported to decrease locomotor activity in rats (Van Hartesveldt et al. 1992).

In the present experiment, administration of 7-OH-DPAT (5 μg) into the core, but not into the shell, decreased maximal responses and total responses. This motor suppressant effect of 7-OH-DPAT is consistent with the results obtained by other investigators (Gilbert et al. 1995; Depoortere et al. 1996). An equivalent dose of 7-OH-DPAT also decreased locomotor activity when administered into the NAS core and shell in the previous experiment (Chapter 7). However, the differential effects of 7-OH-DPAT in the NAS core and shell observed in this experiment may be relevant to the proposal that the NAS core is primarily related to motor function whereas the shell is involved in limbic system function (Deutch et al. 1993). Carlezon and Wise (1996a) reported that the NAS shell is critically involved in rewarding effects of NMDA receptor antagonists after medial forebrain bundle self-stimulation. The NAS shell has also been reported to be preferentially involved in feeding behaviour (Kelley 1999). However, this functional difference may depend on different indices of behaviour, as there is now evidence for equivalent effects of 7-OH-DPAT in the NAS core and shell in the context of spontaneous locomotor activity (Chapter 7).

Administration of the AMPA/kainate receptor antagonist CNQX (0.5 μg) into the NAS core or shell did not alter reward threshold or rate responses. The latter result is

consistent with a lack of locomotor effects of CNQX (0.5 μg) in the NAS core and shell (Chapter 5). Higher doses of CNQX were not tested because of poor solubility of the CNQX:HBC complex in aqueous solution. It has been suggested that, in contrast to the NMDA receptor antagonists, AMPA/kainate receptor antagonists may be inactive in behavioural tests in experimentally naïve rats (Hauber and Andersen 1993; Bubser et al. 1995). Herberg and Rose (1989) suggested that non-NMDA receptor blockade may decrease brain stimulation reward, however the effects of AMPA/kainate receptor antagonists in the NAS core and shell on VTA self-stimulation have not been reported.

Co-administration of CNQX and 7-OH-DPAT into either the NAS core or shell significantly increased the reward threshold and decreased rate responses. These effects of CNQX and 7-OH-DPAT indicate that concomitant blockade of AMPA/kainate receptors and stimulation of D2/D3 dopamine receptors in the NAS may have a synergistic effect on reward. The combined effects of 7-OH-DPAT and CNQX may be consistent with the proposal that the effects of D3 dopamine receptor stimulation are functionally opposite to the postsynaptic actions of D2 dopamine receptor agonists (Diaz et al. 1994; Svensson et al. 1994b; Waters et al. 1993). Carlezon and Wise (1996a) reported that administration of PCP into the NAS shell potentiated medial forebrain bundle brain stimulation reward. These authors suggested that the effect of PCP is mediated by inhibition of medium spiny GABA neurons in the NAS shell. PCP may block dopamine uptake and antagonize NMDA receptors that synergistically decrease output of these neurons. However, similar reward enhancing effects of MK-801 and CPP,

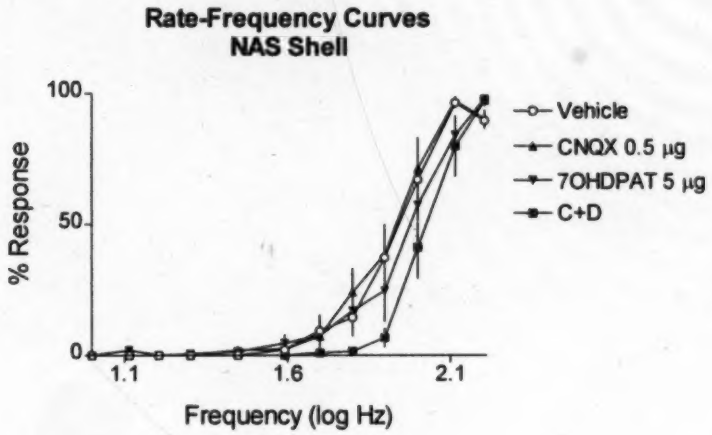
which have negligent effects on the dopamine uptake system, may be difficult to attribute to this hypothesis. In contrast, the D2/D3 dopamine agonist 7-OH-DPAT has been reported to decrease dopamine release in the NAS (Timmerman et al. 1991; Damsma et al. 1993; Gilbert et al. 1995) and to decrease the reward effect of ICSS (Gilbert et al. 1995; Kling-Peterson et al. 1995; Depoortere et al. 1996). Furthermore, in contrast to the NMDA receptor antagonists, non-NMDA receptor antagonists may decrease the reward effect of ICSS (Herberg and Rose 1990). Therefore, it is possible that a combination of CNQX and 7-OH-DPAT may have a specific effect on brain stimulation reward when co-administered into the NAS core and shell.

Several pieces of evidence suggest that D3 dopamine receptors and AMPA/kainate receptors may be involved in reward-related behaviour. Recent studies have suggested that D3-preferring dopamine agonists may block reward-related behaviour in rodents. For example, 7-OH-DPAT reduced amphetamine- or cocaine-induced conditioned place preference (Khroyan et al. 1998; 1999). 7-OH-DPAT decreased cocaine self-administration at doses that were not reinforcing by themselves (Caine and Koob 1993). Partial D3 agonists may inhibit cocaine-seeking behaviour without having intrinsic and primary rewarding effects (Pilla et al. 1999). AMPA/kainate receptor antagonists may also reduce psychomotor stimulant effects and prevent behavioural sensitization. DNQX inhibited behavioural sensitization of amphetamine and cocaine in mice (Karler et al. 1991; 1994). CNQX decreased amphetamine-induced locomotor activity and conditioned reinforcement responding in rats (Burns et al. 1994). The more selective AMPA receptor antagonist NBQX prevented development of cocaine

sensitization in rats (Li et al. 1997).

Recent studies have suggested that repeated administration of psychomotor stimulants may alter the expression of AMPA receptor subunits in the NAS (Lu et al. 1997; Churchill et al. 1999; Kelz et al. 1999). Repeated administration of amphetamine decreased GluR1 and GluR2 expression in the NAS (Lu et al. 1997). Pierce et al. (1996) and Churchill et al. (1999) have reported that repeated administration of cocaine may increase glutamate transmission and GluR1 levels in the NAS only in rats that develop behavioural sensitization. In addition, Kelz et al. (1999) have provided evidence for increased GluR2 expression in the NAS *via* expression of transcription factor ΔFosB following repeated administration of cocaine. These studies suggest that AMPA receptors in the NAS have an important role in the regulation of behavioural sensitization mediated by psychostimulants such as amphetamine and cocaine. Together with previous studies, the present results suggest that possible interactions of D3 dopamine receptors and AMPA receptors in the NAS core and shell may represent a significant focus for research into psychotherapeutic drug action in the context of drug addiction and schizophrenia.

Figure 13.1. Rate-frequency curves and non-linear regression curves related to the NAS shell. CNQX (0.5 μg) and 7-OH-DPAT (5 μg) were microinjected into the NAS shell and rate-responses were measured according to frequency changes. Regression curves were calculated from the rate-frequency curves. C+D: CNQX+7-OH-DPAT Data are means±S.E.M. (n=7)



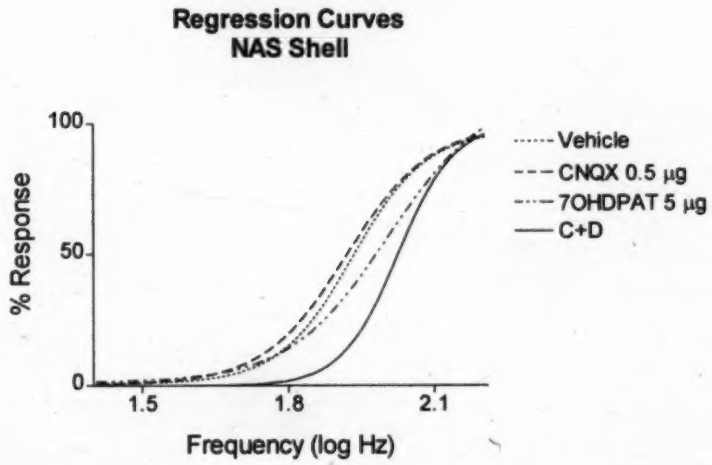
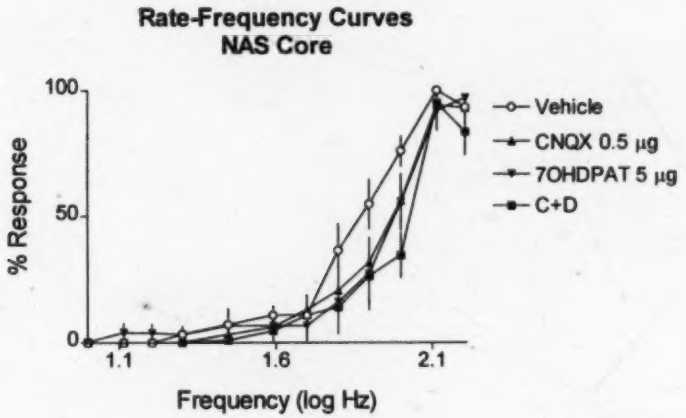


Figure 13.2. Rate-frequency curves and non-linear regression curves related to the NAS core. CNQX (0.5 μg) and 7-OH-DPAT (5 μg) were microinjected into the NAS core and rate-responses were measured according to frequency changes. Regression curves were calculated from the rate-frequency curves. C+D: CNQX+7-OH-DPAT Data are means±S.E.M. (n=6)



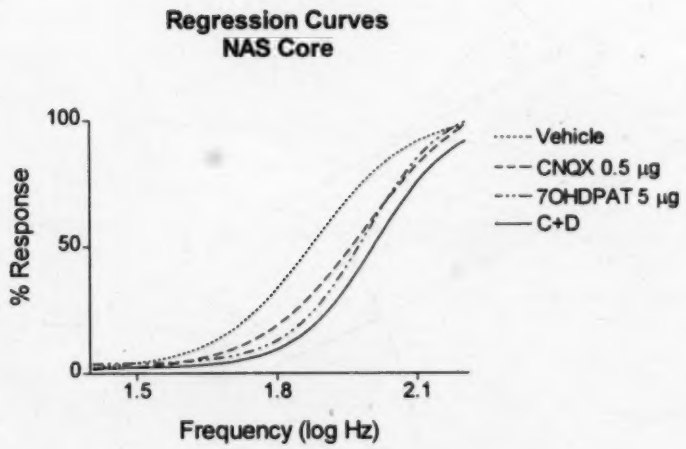


Figure 13.3. Effects of CNQX and 7-OH-DPAT in the NAS shell and core on reward threshold (M50). Vehicle, CNQX (0.5 μg), 7-OH-DPAT (5 μg) and a mixture of CNQX and 7-OH-DPAT were microinjected into the NAS shell (n=7) or core (n=6) and M50 responses were measured. Data are medians±interquartile. *Significant at P<0.05 relative to vehicle. C+D: CNQX+7-OH-DPAT

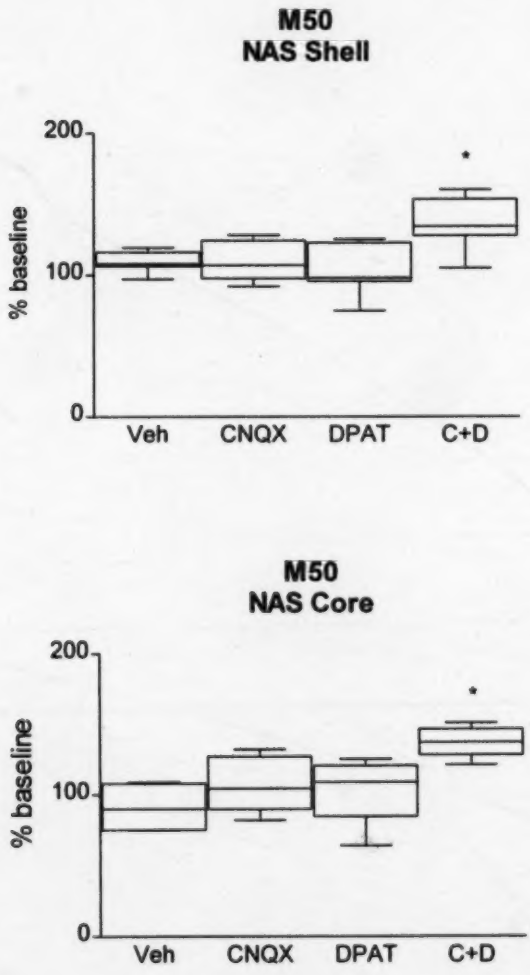


Figure 13.4. Effects of CNQX and 7-OH-DPAT in the NAS shell and core on maximal responses (RMAX). Vehicle, CNQX (0.5 μg), 7-OH-DPAT (5 μg) and a mixture of CNQX and 7-OH-DPAT were microinjected into the NAS shell (n=7) or core (n=6) and RMAX responses were measured. Data are medians±interquartile. *Significant at P<0.05 relative to vehicle. C+D: CNQX+7-OH-DPAT

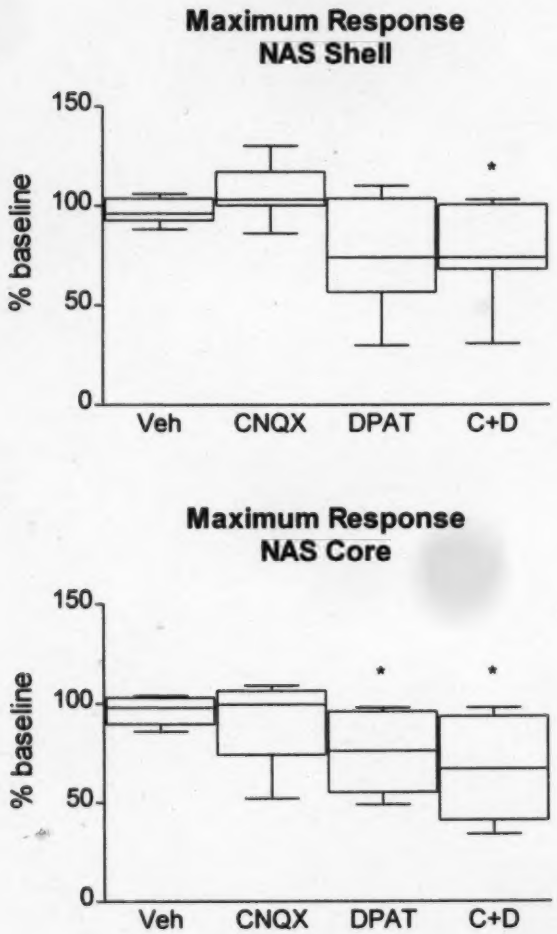


Figure 13.5. Effects of CNQX and 7-OH-DPAT in the NAS shell and core on total responses (TRES). Vehicle, CNQX (0.5 μg), 7-OH-DPAT (5 μg) and a mixture of CNQX and 7-OH-DPAT were microinjected into the NAS shell (n=7) or core (n=6) and TRES responses were measured. Data are medians±interquartile. *Significant at $P<0.05$ relative to vehicle. C+D: CNQX+7-OH-DPAT

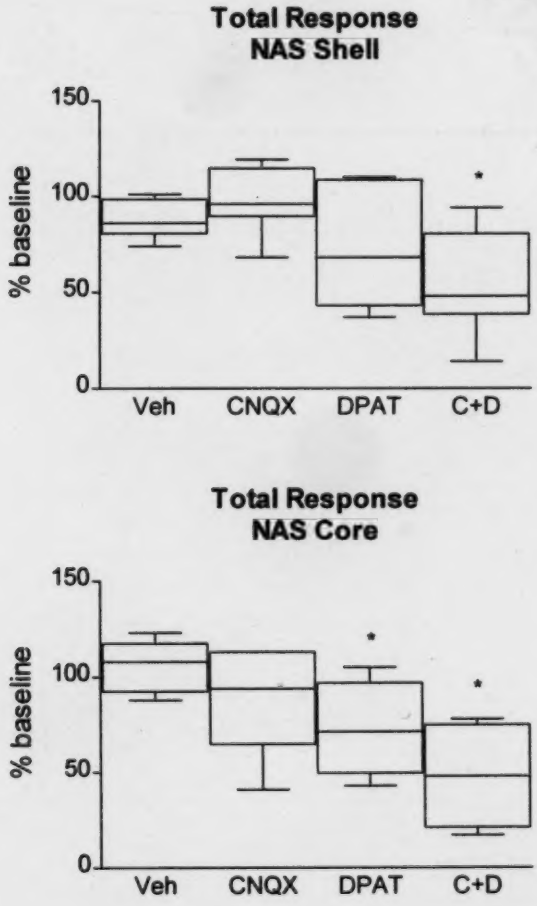


Figure 13.6. Effects of CNQX and 7-OH-DPAT in the NAS shell and core on zero frequency (ZFRE). Vehicle, CNQX (0.5 μg), 7-OH-DPAT (5 μg) and a mixture of CNQX and 7-OH-DPAT were microinjected into the NAS shell (n=7) or core (n=6) and ZFRE responses were measured. Data are medians±interquartile. *Significant at $P<0.05$ relative to vehicle. C+D: CNQX+7-OH-DPAT

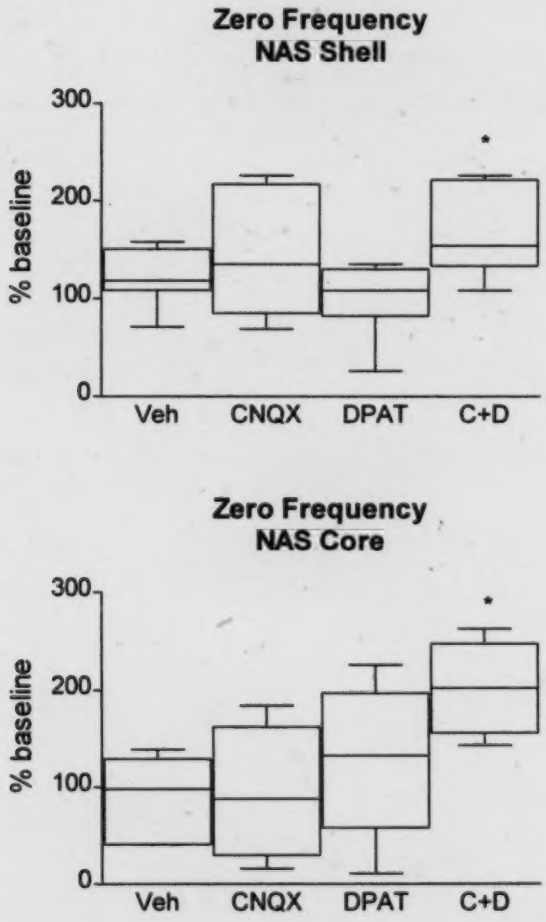


Figure 13.7. Histological location of microinjection sites in the core (O) and shell (●) of the NAS. Numbers represent distance from interaural zero plotted on modified coronal sections from the atlas of Paxinos and Watson (1986).

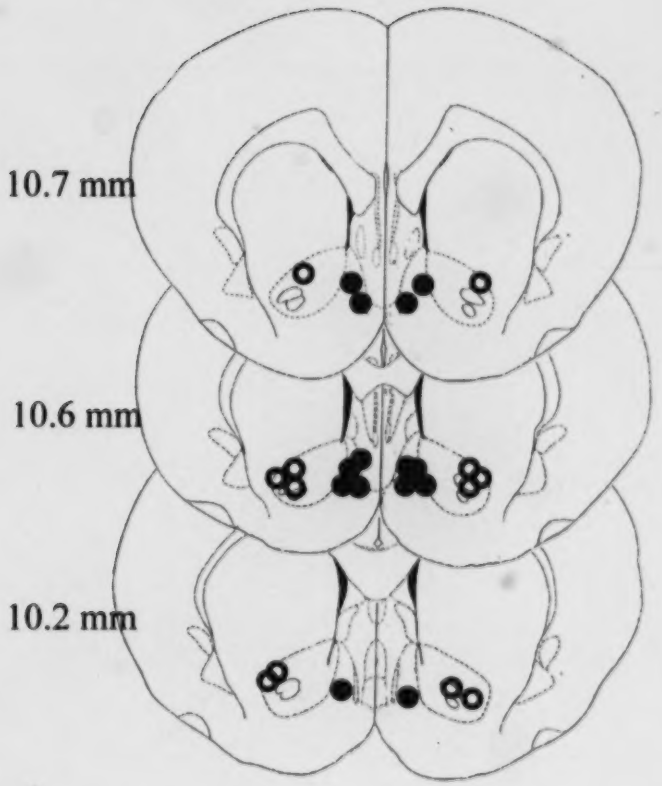
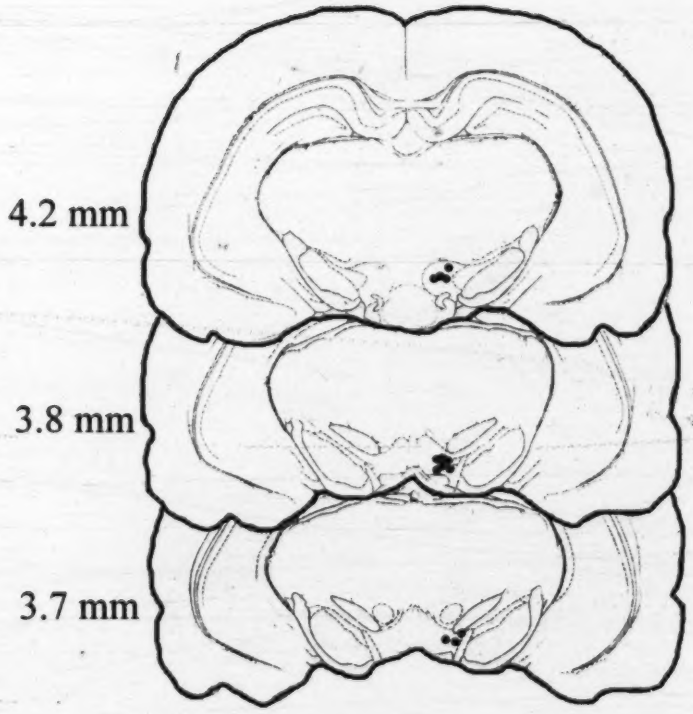


Figure 13.8. Histological verification of electrode placements in the VTA. Numbers represent distance from interaural zero plotted on modified coronal sections from the atlas of Paxinos and Watson (1986).



14. GENERAL DISCUSSION

Interactions between dopamine and glutamate in the NAS have been implicated in the pathophysiology of schizophrenia. These interactions may also play a significant role in the mechanisms of drug addiction in humans. The purpose of this study was to investigate behavioural and neurochemical effects of glutamate- and dopamine-related drugs when directly administered into the NAS core and shell in rats.

In the first part of this study, the effects of the glutamate uptake inhibitor PDC and the NMDA receptor co-agonist glycine on spontaneous locomotion and electrical self-stimulation were examined. These experiments were conducted based on the hypothesis of NMDA receptor hypofunction in schizophrenia. Although administration of PDC into the NAS markedly increased extracellular glutamate levels, spontaneous locomotor activity was not altered by this treatment (Chapter 3). Due to the indirect and non-selective effects of PDC on different subtypes of glutamate receptors, the NMDA receptor co-agonist glycine was examined in the next experiment (Chapter 4). Chronic oral administration of glycine induced small but significant elevation of brain glycine levels. However, chronic glycine treatment did not alter electrical self-stimulation in rats. The lack of effect of glycine on electrical self-stimulation may be relevant to clinical efficacy of glycine therapy improving negative symptoms but not positive symptoms in schizophrenic patients.

In the second part of this study, the interactions of the AMPA/kainate receptor antagonists CNQX and NBQX with indirect or direct dopamine receptor agonists on locomotion and ICSS behaviour were investigated. Due to the heterogeneity of the NAS,

separate groups of animals were used to compare the drug effects between the NAS core and shell. Synergistic interactions between AMPA/kainate receptor antagonists and the D2/D3 dopamine receptor agonist 7-OH-DPAT were found in the NAS core and shell based on measurements of locomotion (Chapter 7) and brain stimulation reward (Chapter 13). This interaction suggests that concomitant blockade of the AMPA/kainate receptors and stimulation of D2/D3 receptors in the NAS may synergistically reduce locomotion and increase reward thresholds in rats. Therefore this interaction may provide useful information for understanding motivation and reward-related behaviour, and the pathophysiology of schizophrenia.

Although dysfunction of NMDA receptors has been proposed in the pathophysiology of schizophrenia, AMPA/kainate receptors may also play a significant role because of fast-synaptic transmission to activate NMDA receptors on the postsynaptic membrane. Other studies have reported differential behavioural effects of AMPA/kainate receptor-related drugs compared to NMDA receptor-related compounds (Danysz et al. 1994; Bubser et al. 1995; Stephens and Cole 1996). Intra-NAS administration of the AMPA/kainate receptor antagonist CNQX (0.25-0.5 μg) did not alter spontaneous locomotor activity in this study (Chapter 5). This result is consistent with previous reports suggesting that AMPA/kainate receptor antagonists are behaviourally inactive in rodents (Hauber and Andersen, 1993; Danysz et al. 1994; Bubser et al. 1995). Bubser et al (1995) suggested that AMPA/kainate antagonists may be suitable therapeutic agents for anticonvulsive and neuroprotective actions without having significant motor side effects. Svensson (2000) further suggested that AMPA/KA

receptor antagonists may have atypical antipsychotic properties based on a conditioned avoidance response study.

In contrast, other studies have reported a locomotor stimulant effect (Burns et al. 1994) and a disruption of pre-pulse inhibition (Wan and Swerdlow, 1996) following intra-NAS administration of CNQX. Burns et al. (1994) measured locomotor activity for 2 hours following administration of CNQX (0.48 μg) into the NAS. Animals were habituated to photocell cages equipped with 2 lower and 4 upper photobeams for measuring horizontal and vertical activity for 2 hours prior to microinjection. In the present study, the photobeam boxes were equipped with 12 × 12 horizontal and 12 vertical photobeams to monitor spontaneous locomotion at each 5-min interval. This procedure is more sensitive for monitoring spontaneous activity with detailed temporal analysis than the experiment reported in the study of Burns et al. (1994). Furthermore, CNQX (0.5 μg) was tested in a series of experiments in this study but no significant stimulant effect was found. The differential effects of CNQX observed between the study of Burns et al. (1994) and the present study may be due to a different habituation procedure (same day vs. previous days) or different photocell cages used.

The indirect dopamine agonist amphetamine (1.25-5 μg), when administered into the NAS shell, dose-dependently increased locomotor activity (Chapter 5). This stimulant effect was observed following microinjection of amphetamine into the NAS shell but not the core. This result is consistent with previous studies that reported site-selective action of amphetamine in the NAS sub-regions on locomotor activity (Essman et al. 1993; Heidbreder and Feldon 1998). These authors demonstrated greater responses to amphetamine when microinjected into the shell than core of the NAS. These results

suggest that co-activation of D1-like and D2-like dopamine receptors in the NAS shell may mediate behavioural stimulation indicated by amphetamine. In accord with these results, co-administration of the D1-like agonist SKF 38393 and the D2-like agonist quinpirole into the NAS shell, but not the core, induced marked behavioural stimulation in this study (chapter 12).

Due to the site-selective action of amphetamine observed in the shell, the effect of CNQX on amphetamine-induced hyperactivity was investigated in the shell of the NAS (Chapter 5). CNQX (0.5 μg) and amphetamine (5 μg), alone or in combination, were administered into the NAS shell and locomotor activity was monitored for 60 min. CNQX did not alter overall amphetamine-hyperactivity (60 min). However there was a statistically significant interaction of CNQX × amphetamine × time as analyzed by ANOVA with repeated measures. The local time course data at each 5-min interval revealed that the combination of CNQX and amphetamine decreased amphetamine-induced hyperactivity during the first 20 min of the testing period. This finding is consistent with observations by others that suggest inhibitory effects of AMPA/kainate receptor antagonists on psychostimulant-induced hyperactivity (Burns et al. 1994; Kaddis et al. 1993). Similar to the present result, the inhibitory effect of CNQX on amphetamine-induced hyperactivity was only observed in the first 30 min following microinjection in the Burns et al. (1994) study. Even though it is tempting to hypothesize that blockade of AMPA/kainate receptors in the NAS may inhibit psychostimulant-induced locomotor hyperactivity, it is difficult to explain this interaction because of the relatively small and transient effect of CNQX and the non-specific (indirect) effects of amphetamine on

different subtypes of dopamine receptors. Therefore, CNQX was tested with direct D1 and D2/D3 dopamine agonists in the NAS core and shell in further experiments.

The interaction between dopamine and glutamate in the NAS may depend on the relative involvement of D1-like and D2-like dopamine receptors. Svensson et al. (1992) suggested differential effects of the NMDA receptor antagonist MK-801 on responses to D1 and D2 dopamine agonists using monoamine-depleted mice. Kaddis et al. (1993) also reported an inhibitory effect of the AMPA/kainate receptor antagonist DNQX on hyperactivity induced by a combination of SKF 38393 and quinpirole in the NAS. However, the effects of DNQX on separate applications of SKF 38393 and quinpirole were not reported in that study. Therefore, in the present study, the effect of CNQX was compared with the D1 agonist SKF 38393 or the D2/D3 agonists (7-OH-DPAT or quinpirole). Administration of SKF 38393 into the NAS core and shell increased locomotion whereas 7-OH-DPAT or quinpirole decreased locomotion. These effects were observed both in the core and shell of the NAS, and there was no significant difference between these regions. Although SKF 38393 induced more prominent late hyperactivity when administered in the shell than in the core, 60 minutes of total activity analysis was not different between these regions. Other studies have shown a stimulant effect of SKF 38393 following microinjection into the NAS (Dreher and Jackson, 1989; Phillips et al. 1995), but no regional analysis of SKF 38393 within the NAS has been reported. Microinjection of the D2/D3 dopamine agonist, quinpirole (3 μg), into different striatal regions decreased locomotion and rearing, but there were no regional differences among the NAS or the ventrolateral or anterodorsal striatum (Canales and Iversen, 1998). The site-independent effects of D1 and D2/D3 agonists in the NAS core and shell observed in

this study are not likely due to drug diffusion between these regions because co-administration of SKF 38393 and quinpirole into the NAS shell, but not the core, selectively increased locomotion.

When co-administered with 7-OH-DPAT (5 μg), CNQX (0.5 μg) potentiated the locomotor suppressant effect of 7-OH-DPAT in the NAS core and shell (Chapter 7). However, this interaction was not observed when CNQX was tested in combination with quinpirole (5 μg). These results suggest that blockade of AMPA/kainate receptors in the NAS may have differential effects on locomotion depending on relative stimulation of D3 and D2 receptors. Although *in vivo* selectivity of 7-OH-DPAT for the D3 receptor is controversial, a number of studies have used 7-OH-DPAT as a D3-preferring agonist in behavioural studies (see Levesque 1996; Shafer and Levant, 1998). Similar to the present study, some studies have shown differential effects of 7-OH-DPAT and quinpirole on responses to glutamate- and dopamine receptor-related drugs (Dall'Olio et al. 1997; Maj et al. 1999). Systemic administration of the NMDA receptor antagonist MK-801 counteracted hypoactivity induced by 7-OH-DPAT but not by quinpirole (Dall'Olio et al. 1997). Maj et al. (1999) also reported that a D3 receptor-preferring antagonist, nafadotride, counteracted locomotion induced by 7-OH-DPAT but not by quinpirole. The selective interaction of CNQX with 7-OH-DPAT but not with quinpirole observed in this study may have been due to partial D3 receptor agonism of 7-OH-DPAT. Although these agonists have similar affinity for D2 receptors, 7-OH-DPAT is two times more selective for D3 receptors than quinpirole in functional studies (Sautel et al. 1995). Therefore, it is likely that D3 receptors are involved in this interaction of CNQX and 7-OH-DPAT in the NAS core and shell. Moreover, recent studies have proposed that the D3 receptors are

implicated in the pathophysiology of schizophrenia and cocaine addiction (Harrison 1999; Pilla et al. 1999; Mash and Staley 1999; Schwartz et al. 2000).

In addition to D2/D3 dopamine receptors, D1 dopamine receptors may play a significant role in locomotion and reward-related behaviour (Sutton and Beninger 1999). For this reason, CNQX (0.5 μg) was tested in combination with the D1 dopamine agonist SKF 38393 (5 μg) in a locomotor activity study (Chapter 10). CNQX did not alter SKF 38393-hyperactivity when co-administered into the NAS core and shell. Due to the late onset of hyperactivity induced by SKF 38393, SKF 38393 followed by CNQX was microinjected into the NAS shell with a 25 min interval and locomotor activity was monitored (Chapter 11). CNQX did not alter late onset SKF 38393-induced hyperactivity. These results suggest that locomotor stimulation induced by stimulation of D1 receptors in the NAS may not be affected by the blockade of AMPA/kainate receptors. Consistent with this result, Keefe and Gerfen (1999) demonstrated that CNQX did not affect immediate early gene expression induced by SKF 38393 in the rat striatum. Based on these results, it was hypothesized that blockade of AMPA/kainate receptors and stimulation of D3/D2 receptors, but not D1 receptors, in the NAS core and shell may have a synergistic effect on locomotion. This hypothesis may have important implications for interactions between dopamine and glutamate in the mesolimbic system in relation to schizophrenia and drug addiction in humans.

Stimulation of both D1-like and D2-like dopamine receptors in the NAS may have a synergistic effect on locomotor behaviour (Waddington et al. 1995; Phillips et al. 1995). A similar synergistic effect on locomotor activity was reported following co-administration of SKF 38393 and 7-OH-DPAT into the NAS (Barik and Beaurepaire

1998). Although previous studies have demonstrated synergistic interaction of D1-like and D2-like receptors on locomotor activity, very few studies have distinguished the differences between the NAS core and shell in this context. In the present study (Chapter 12), SKF 38393 and quinpirole were co-administered into the NAS core or shell and locomotor activity was monitored. This combination synergistically increased locomotor activity when co-administered into the NAS shell, but not into the core. This result suggests that simultaneous activation of D1 and D2/D3 receptors in the NAS shell is necessary to stimulate locomotor behaviour. In accord with this finding, recent studies suggested differential effects of D1-like and D2-like interaction in the core and shell of the NAS on rotation behaviour and jaw movement in rats (Koshikawa et al. 1996; Kitamura et al. 1999).

At a cellular level, D1 and D3 receptors may co-exist on a single neuron in the NAS shell and show synergistic effects on c-fos mRNA expression (Ridray et al. 1998). Seeman et al. (1989) proposed that abnormal interactions between D1-like and D2-like dopamine receptors may be relevant for the pathophysiology of schizophrenia. Furthermore, Schwartz et al. (1998) suggested that an imbalance between D1 and D3 receptors in the NAS shell may play a role in schizophrenia. The synergistic effects of SKF 38393 and quinpirole in the NAS shell observed in this study may be relevant to this hypothesis. However, it is difficult to interpret the mechanisms of the D1 and D2/D3 receptor interaction in the NAS shell because of various factors, including separate or co-localization of these receptors, involvement of GABA interneurons and indirect or direct output pathways to the basal ganglia.

Both the mesolimbic dopamine system and the corticolimbic glutamate system have been implicated in reward-related behaviour and drug addiction (Mogenson et al. 1980; Self and Nestler 1995; Wise 1996b; Pierce and Kalivas 1997; Koob et al. 1998; Bardo 1998; Schultz 1998; Berridge and Robinson 1998). The NAS may play a critical role in mediating reward-related behaviour because this nucleus receives dopaminergic and glutamatergic input from the VTA and the prefrontal cortex, respectively. In the present study (Chapter 13), the effects of CNQX (0.5 μg) and 7-OH-DPAT (5 μg) on brain stimulation reward were investigated based on synergistic effects observed in the previous locomotor activity experiment. Microinjection of CNQX into the NAS core or shell did not alter reward threshold or response rate in ICSS. Microinjection of 7-OH-DPAT into the core, but not the shell, decreased response rate; reward threshold was not affected by injection of the drug into either the core or shell of the NAS. The effects of systemic administration of 7-OH-DPAT on reward threshold are not consistent with observations made in previous studies (see Shafer and Levant, 1998) and no regional analysis within the NAS sub-regions has been reported. This experiment found that co-administration of CNQX and 7-OH-DPAT into the core or shell significantly increased reward threshold and reduced response rates. Decreased response rate is consistent with the locomotor suppressant effect of this combination shown previously (Chapter 7). Reward thresholds were measured using a rate-frequency curve-shift paradigm, which is relatively independent of response rate. Based on these findings, it was hypothesized that concomitant stimulation of D2/D3 receptors and blockade of AMPA/kainate receptors in the NAS sub-regions may reduce brain stimulation reward in rats.

In vivo techniques such as intracranial microinjection and *in vivo* microdialysis have greatly enhanced the understanding of neurotransmitter-specific neuronal circuitry in the brain. For example, the intra-NAS microinjection and intra-VTA self-stimulation methods used in this study were able to test drug effects on brain stimulation reward in the mesolimbic dopamine system in rats. *In vivo* microdialysis combined with HPLC analysis was used to detect various neurochemicals in the extracellular space of the brain while animals are freely moving. However these *in vivo* techniques also have some limitations.

Although intracranial microinjection of a drug into a specific brain region has been extensively used to study drug effect on various types of behaviour, this technique has some limitations as mentioned in previous studies (Carvey et al. 1994; McBride et al. 1999). First, selectivity of a drug to specific receptors *in vivo* may be significantly different from the data obtained from *in vitro* ligand binding studies. 7-OH-DPAT may be 30-100 times more selective for D3 receptors than D2 receptors in a ligand binding study (Levesque et al. 1992), but this selectivity decreases to 4-7 times in functional assays (Sautel et al. 1995). Second, anatomical selectivity following microinjection of a drug needs to be verified. When a drug solution is infused into the brain, diffusion starts to occur in a circular gradient over the infusion site. Because anatomical structures are generally not circular, this may cause either incomplete or nonspecific drug exposure to the surrounding structure. Therefore, it is necessary to use an injection volume of 0.5 μl or less and include careful neuroanatomical experiments as a positive control.

In this study, the NAS core and shell were investigated using an intracranial microinjection technique. Because the NAS core and shell are about 1.5 mm apart, each

region can serve as neuroanatomical control for the other. Third, repeated microinjections may cause local non-specific tissue damage, which could affect locomotor behaviour. For this reason, approximately four microinjections were delivered to each animal, with a three-day interval between each microinjection in most experiments. The schedules of microinjections were counter-balanced using a Latin square design to minimize confounding drug effects of previous injections. Consistent drug responses were obtained with this procedure following repeated microinjections in this study.

Recent studies on regional differences in neurochemical organization within the core and shell sub-regions of the NAS have revealed an even finer level of complexity that may lead to a re-evaluation of the data that are currently available. The NAS core exhibits a patch-striosome dichotomy similar to that of the caudate putamen and the NAS shell exhibits at least three different compartments based on tract-tracing and immunostaining studies (Heimer et al. 1997; Groenewegen et al. 1999; Zahm 1999). Further elucidation of this organization within the NAS core and shell sub-regions may be necessary for understanding differential interactions of dopamine and glutamate projections to the NAS beyond the simple core and shell dichotomy.

Intracranial self-stimulation (ICSS) has been widely used to study reward-related drug effects and antipsychotic drug action, but this method also has some limitations. First, lever-pressing responses in ICSS cannot distinguish between performance and reward effects. To overcome this problem, numerous studies have developed different reward threshold measures which are relatively rate-independent, such as choice measures, response pattern analysis and threshold measures (see Greenshaw and Wishart 1987; Liebman 1989; Wise and Rompre 1989). Second, ICSS may cause an energizing

effect in animals, which may increase performance and be unrelated to reward effects. However, this effect may be more significant in lateral hypothalamus stimulation than in VTA stimulation. Third, ICSS may cause non-specific stimulation of other neurotransmitter neurons existing in the surrounding area of stimulation. Among various regions involved in reward effects of ICSS, the VTA was chosen in this experiment because this region contains mainly dopaminergic neurons projecting to the NAS sub-regions.

Although recent advances in *in vivo* microdialysis and HPLC methods permit the analysis of neurochemical changes in freely moving animals, there are some limitations to this technique. First, *in vivo* microdialysis has poor temporal resolution since several minutes are required to collect dialysates in animals. Considering neurotransmitter release and uptake in the synapse occur within milliseconds, microdialysis may only reflect gross changes in extracellular neurotransmitter levels around the microdialysis probe (Adell and Artigas 1998). Second, anatomical selectivity is difficult to achieve in small brain areas because of the relatively large diameter of the dialysis probe and constant diffusion of the drug solution from the dialysis probe to the surrounding tissue. The NAS core and shell are adjacent areas and it is difficult to distinguish these regions with conventional microdialysis probes for these reasons. Therefore, the microdialysis data obtained in this study were related to the NAS as a unitary structure. Third, in the case of direct infusion of a drug through the dialysis probe, it is difficult to calculate the actual concentration of the drug which affects the tissue around the infusion site. Several factors may affect administration of a drug through the dialysis membrane, including the diffusion coefficient, drug concentration, drug interaction with membrane, temperature,

uptake of the drug in vascular compartment and binding to receptor or membrane proteins (Benveniste 1989).

NMDA receptor antagonists and AMPA/kainate receptor antagonists may have different effects on behaviour. It is possible that non-competitive NMDA receptor antagonists induce psychotomimetic symptoms (Javitt and Zukin 1991) whereas AMPA/kainate receptor antagonists may produce antipsychotic effects (Svensson 2000). In animal studies, using systemic administration, NMDA receptor antagonists stimulate locomotor activity while AMPA/kainate receptor antagonists do not significantly affect locomotor behaviour (Hauber and Anderson, 1993; Bubser et al. 1995). These authors also reported that the locomotor-stimulant effect of the NMDA receptor antagonist MK-801 was attenuated by the AMPA/kainate antagonist, GYKI 52466, which did not affect motor activity when given alone. The differential effects of NMDA and AMPA/kainate receptor antagonists on behaviour is intriguing because both receptors are involved in fast synaptic glutamate transmission in the synapse.

However, recent studies have suggested that these differential effects may be attributable to interactions between glutamate and dopamine in the mesocortico/mesolimbic dopamine system. Moghaddam et al. (1997), using *in vivo* microdialysis, reported that application of CNQX into the prefrontal cortex blocked dopamine release induced by systemic administration of the NMDA receptor antagonist ketamine. These authors also observed that another AMPA/kainate antagonist LY 293558 counteracted impairment of the spatial delayed alternation in rats induced by ketamine. Systemic administration of ketamine may indirectly activate AMPA/kainate receptors in the prefrontal cortex by elevating glutamate release and this effect can be blocked by

AMPA/kainate antagonists. This facilitatory effect of ketamine on glutamate release in the prefrontal cortex may be due to blockade of presynaptic NMDA receptors (Smirnova et al. 1993; Wang and Thukral 1996) or disinhibition of GABAergic inputs to glutamate-containing neurons (Moghaddam et al. 1997). Therefore, under certain conditions, it is possible that activation of AMPA/kainate receptors may account for some of the behavioural effects induced by the NMDA receptor antagonists.

In addition, Mathé et al. (1998) reported that administration of CNQX into the VTA blocked locomotor hyperactivity and dopamine release in the NAS induced by systemic administration of MK-801. The stimulant actions of MK-801 on the mesolimbic dopamine system are likely mediated by indirect mechanisms involving the VTA. This mechanism is not well established, however these authors have suggested that systemic administration of MK-801 elevates extracellular glutamate levels in the VTA which results in dopamine release in the NAS *via* activation of AMPA/kainate receptors in the VTA. In another study, Mathé et al. (1999) reported that a novel AMPA receptor antagonist, LY 326325, reduced a conditioned avoidance response without affecting escape behaviour which implicates potential antipsychotic efficacy of this compound. Based on these studies, the behavioural effects of NMDA receptor antagonists may not only involve NMDA receptor blockade but also reflects an increased activation of AMPA/kainate receptors in the mesocortico/mesolimbic dopamine system. Therefore, in contrast to the NMDA receptor antagonists which induce psychotomimetic symptoms, the AMPA/kainate receptor antagonists may have antipsychotic efficacy alleviating some symptoms of schizophrenia.

An interaction between CNQX and 7-OH-DPAT was found in this study. Co-administration of CNQX and 7-OH-DPAT into the NAS core or shell reduced spontaneous locomotor activity and brain stimulation reward. This result suggests a functional relationship between AMPA/kainate receptors and D2/D3 dopamine receptors in the NAS in the context of movement and reward-related behaviour. The NMDA receptors may not be involved in this interaction because the more selective AMPA receptor antagonist NBQX also had a similar action when tested with 7-OH-DPAT. In addition, D3 dopamine receptors may play an important role in this interaction. Another D2/D3 receptor agonist, quinpirole, did not induce synergistic effects when tested with CNQX. Although *in vivo* selectivities of 7-OH-DPAT and quinpirole to D2 and D3 dopamine receptors are not consistent, it has been suggested that 7-OH-DPAT is approximately twice as selective to D3 receptors as quinpirole in functional studies (Sautel et al. 1995). Therefore, it is possible that microinjection of 7-OH-DPAT into the NAS may affect a different sub-population of D3 receptors compared to the equivalent dose of quinpirole. Recent studies have proposed that partial D3 receptor agonists, which have fewer motor and addictive side effects, may be beneficial in treating cocaine addiction and schizophrenia (Pilla et al. 1999; Koob and Caine 1999; Childress and O'Brien 2000; Schwartz et al. 2000). In accord with these hypotheses, 7-OH-DPAT did not alter brain stimulation reward threshold when microinjected into the NAS core and shell in this study (Chapter 13). However, the combination with CNQX significantly reduced reward and motor effects. Therefore, in addition to the D3 dopamine receptor hypothesis, the interactive effects of AMPA/kainate receptors and D3 dopamine receptors

in the NAS found in the present study may provide further information for understanding the pathophysiology of schizophrenia and the mechanisms of drug addiction in humans.

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